

What do we know about the malaria vaccines?

Editorial

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Of the five malaria-causing species, *Plasmodium falciparum* is the most dangerous especially for infants. Unfortunately because children below five years of age have not yet gained any degree of immunity, they are severely affected with possible fatal complications. This also applies to naïve individuals infected for the first time. Infection does not confer solid immunity for indigenous residents of endemic areas, but allows an incomplete amount of acquired immunity resulting in less severe future attacks of malaria. However, even this naturally acquired partial immunity does not last unless the individual is continuously exposed to re-infection. Research for attaining more positive protection focused on preparation of vaccines from multiple phases in the life cycle of *Plasmodium* including attenuated whole organisms and recombinant proteins. The present editorial outlines different trials to obtain a successful malaria vaccine and underlines two malaria vaccines with promising outcome: a PfSPZ vaccine composed of *P. falciparum* sporozoites attenuated by irradiation, and manufactured by Sanaria Inc. and the recombinant fusion proteins formula (RTS,S/AS01) constructed by GlaxoSmithKline (GSK), commercially known under the trade name 'Mosquirix™' (Glaxosmithkline plc, Brentwood, UK).

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The humongous toll of 216 million cases and about 445,000 deaths due to malaria, was reported by the WHO in 2017 with over 90% occurring in Sub-Saharan Africa^[1]. As early as 2002 analysis of the *P. falciparum* genome sequence clone 3D7 revealed that the 23-megabase nuclear genome consisted of 14 chromosomes, encoding about 5,300 genes, mostly specialized in immune evasion and host parasite interactions^[2]. The sequencing of the *P. falciparum* genome encouraged researchers to consider development of different vaccine strategies especially in view of the emergence of resistance to drugs and insecticides. This led researchers to propose different vaccine protocols against different parasite stages.

In the interest of the vertebrate host's immune response, vaccines options were directed to the production of anti-parasitic and anti-toxic immunity. The former involved the humoral and cell mediated immune responses of the host. The latter was concerned with production of a therapeutic vaccine that would reduce associated cerebral symptoms by inhibition of TNF- α .

It was considered that the choice of a vaccine should basically target a collection of antigens to produce antibodies against different developmental stages of the parasite. Consequently studies opted on a vaccine that would effectively stimulate the host immune response to parasite invasion as well as increase the clearance of parasites and simultaneously reduce the symptoms. Accomplished vaccines were intended to target the various stages of the *Plasmodium* parasite: sporozoites; hepatic stages (merozoites, shizonts, hypnozoites); and erythrocytic stages (rings, trophozoites, schizonts, gametocytes). Accordingly, three main types of vaccines were considered for targeting the different

stages of the parasites' life cycle: vaccines for pre-erythrocytic stages to decrease probability of infection; vaccines for erythrocytic stages to control the infection and minimize pathological effects; vaccines to block transmission and prevent infected mosquitoes from spreading the disease^[3]. Current malaria vaccines under preclinical development or in clinical phase trials were recently summarized to include pre-erythrocytic stages (radiated and genetically attenuated and recombinant subunit sporozoites); blood stages (chemically attenuated liver-stage and blood-stages, subunit merozoite protein and merozoite adhesin required for erythrocyte invasion); mosquito stages (pre-fertilization and post-fertilization subunit antigens, and subunit compatible sequences proteins that allow the parasite to evade the *Anopheles gambiae* immune system)^[4].

Being the first stage to come in contact with the vertebrate host, early attempts for production of protective immunity in the host was directed against the sporozoites. In an early trial, research focused on the use of live irradiation attenuated sporozoites; but although immunity was conferred, the vaccine was not cost effective and could not be implemented on a large scale because of difficulty in procuring adequate numbers of sporozoites. Later on, immunization of volunteers was attempted by exposure to sporozoites transferred from bites of irradiated mosquitoes which conferred immunity by preventing development of hepatic schizonts and infection of red blood cells. However, this vaccine was considered impractical for large scale immunization of susceptible individuals, because the sporozoites must be injected alive either by the bite of irradiated infected mosquitoes, or by intravenous injection. This limited its development as a vaccine.

Recent attempts succeeded in producing the candidate vaccine, *PfSPZ*. That too, is based on sporozoites attenuated by irradiation so that they can only invade hepatocytes without further replication and are able to induce cellular immune responses of CD8⁺ T cells producing IFN γ that prevent future malaria infections. It was first produced as a liquid nitrogen-stored vaccine, which limited its large scale use in Africa^[5]. Updated research on this vaccine, Sanaria®*PfSPZ*, vaccine is continued by Sanaria Inc. with the aim of obtaining a marketing license to be followed by commercial production and implementation in geographically described areas. It is to be implemented in a 15-year elimination and control project called the Bioko Island Malaria Control Project (BIMCP) by Medical Care Development International (MCDI) and other partners in Bioko Island in Sub Saharan Africa^[6].

Another course of research focused on a circumsporozoite protein, CSP, involved in sporozoite motility and invasion. It forms a dense coat surrounding the parasite and is expressed on surfaces of sporozoites and early exo-erythrocytic hepatic stages. Several attempts were made to deploy it for induction of relevant protective immune responses. One of those early attempts turned to immunization with the recombinant [NANP]19-5.1 protein expressed in *E. coli*; composed of 19 repeats of [NANP] peptide from CSP and the protein (5.1); and is transported by schizonts to the red blood surface. It was considered successful having produced 12 weeks protection against *P. falciparum*. The drawback was that it contained only 20% peptide and did not contain any immunodominant T-cell epitopes^[7]. Later, the NYVAC-*Pf7* vaccine was introduced as an attenuated poxvirus-vectored candidate devised to include seven *P. falciparum* antigenic genes covering different stages of the life cycle with promising results encouraging further research. This multi-stage vaccine included: two sporozoites antigens, the CSP and the sporozoite surface protein 2 (*PfSSP2*); one from liver stage (LSA1); three from the erythrocytic stages (merozoite surface protein 1, serine repeat antigen, and an apical membrane antigen, AMA-1); and one sexual stage antigen (25-kDa *Pfs25*). While experimentally, the CSP, *PfSSP2*, MSP1, *Pfs25* antigens elicited prominent specific cell mediated immune responses in humans the antibodies responses were poor^[8]. Additionally, a vaccine based on a recombinant CSP, covalently bound to a purified *Pseudomonas aeruginosa* toxin (A9), was found to produce antibodies that prevent invasion of hepatocytes and a cellular response to enable the destruction of infected hepatocytes. But in its primary form this protein (Asn-Ala-Pro15Asn-Val-Asp-Pro) 2-Leu-Arg (R32LR) proved to be of low immunogenicity^[9]. Later a viral combined vaccine targeting reticulocyte binding protein homolog 5, *PfRh5*, was found to bind to a red blood cell surface receptor known as 'basigin' that permits the invasion of the red cells. It produced a low antibody response in naturally exposed individuals^[10].

The erythrocyte membrane protein-1 (*PfEMP-1*) are a family of antigens exported by the parasite to the red cell surface. *PfEMP1* vaccine was introduced as one of the multiple polymorphic proteins that constitute variant surface antigens controlling parasite transmission, survival, and virulence. It is synthesized by schizogonic stages and expressed on membrane surfaces of *P. falciparum* infected erythrocytes. They are composed of large (200-350 kDa) molecules concentrated in the electron dense protrusions or knobs on the surface of parasitized erythrocytes, recognized as Maurer's clefts in stained parasites, and are the parasite's major cytoadherence ligand and virulence factor. By mediating the binding of infected erythrocytes to the endothelial lining of blood vessels, it constitutes the major parasitic protein involved in the pathology of severe malaria. These variants are expressed one at a time on the infected erythrocyte and the parasites can switch from one to the other, which facilitates sequestration of infected erythrocytes in different tissues. The two most important clinical conditions induced by these variants are cerebral and placental involvement. Trials considered it as a vaccine target because it elicits protective immunity by specific antibodies that significantly decreased the risk of developing symptomatic malaria. Cyto-adhesion and disease outcome by *P. falciparum* was reviewed by Smith^[11]. *PfSEA-1* is composed of a *P. falciparum* schizont egress antigen-1 that produces antibodies to deter rupture of parasites from the infected red blood cells, thus preventing the continuation of their erythrocytic life cycle. Specific antibodies induced in experimentally infected mice interrupted rupture of schizonts consequently decreasing parasite replication. Research on this issue considered that obstruction of schizont egress may add to the effectiveness of other vaccines targeting RBC and hepatocyte invasion^[12].

To induce more potent humoral and cell mediated immune responses, relevant approaches call for consideration of DNA based vaccines as a source of maintained protein expression of multiple antigens from both pre-erythrocytic and erythrocytic stages. A number of DNA vaccines were effective in animal models and are consequently undergoing clinical trials^[13]. Besides vaccination projects, another approach which may prove to enhance control of malaria in endemic areas is directed to application of nano-biotechnology for patient therapy and vector combat. Successfully used compounds include lipids, proteins, nucleic acid and metallic nanoparticles^[14].

As with other vaccine candidates, the development of a vaccine targeting the pre-erythrocytic sporozoite and liver stages (RTS,S), went through preclinical research in animal models. This was followed by phases I–III clinical trials in humans, to determine its capability, immunogenicity and safety before implementation in hyperendemic areas. Promising results encouraged the construction of a recombinant fusion proteins

formula (RTS,S/AS01) by GlaxoSmithKline (GSK) in 1987 followed by several trials up to 2001. Its Phase III trial began in May 2009 including 15460 children in seven countries in sub-Saharan Africa (Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and the United Republic of Tanzania) and was concluded in 2014. Final results were accepted in 2015 as the most promising vaccine for providing relevant protection. This recombinant vaccine is composed of the repeat and T-cell epitope in the pre-erythrocytic CSP protein of *P. falciparum* combined with an HBsAg viral envelop protein with the addition of the chemical AS01 as adjuvant^[15]. This combination avoided the problem of CSP poor immunogenicity, eliciting humoral and cellular immune responses that prevent sporozoites from infecting liver cells. Having gained acknowledgement for implementation the vaccine was commercially produced under the trade name 'Mosquirix™' (Glaxosmithkline plc, Brentwood, UK). Production is by GSK and the Walter Reed Army Institute of Research (WRAIR), and is supported by partial funding by the PATH Malaria Vaccine Initiative^[15] and the Bill and Melinda Gates Foundation^[16]. In April, 2019 a pilot vaccination project was initiated in Malawi and Ghana, to be followed by western Kenya in September 2019^[17]. A 25 µg dose is administered intramuscularly in the deltoid muscle to two groups of children aged 6-14 weeks and 5-17 months in three doses at one month intervals, followed by the fourth and most important booster dose after 18 months, to obtain full protection. So far the vaccine proved its efficiency and safety but still there are other factors to be considered in order to prove its appropriateness for wide scale implementation in endemic areas. These include duration of immunity and assessment of cost-effectiveness of clinical trials to evaluate the immune response, production and marketing. A phase IV research evaluation by GSK is intended to cover more data on vaccine efficiency and side effects of long term implementation of the vaccine in the phase III. Recently in September 2019 the WHO published a report covering answers for all relevant questions concerning the vaccination project^[18,19].

REFERENCES

1. World Health Organization. World Malaria Report 2017. Geneva: License: CC BY-NC-SA 3.0 IGO.
2. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, *et al.* Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 2002; 419(6906):498-511.
3. Shurkin J. There are 3 types of potential malaria vaccines and 2 might be bad ideas. www.insidescience.org/news/2015.
4. 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.
5. 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.
6. Butler D. Promising malaria vaccine to be tested in first large field trial. *Nature Magazine News* 2019; DOI: 10.1038/d41586-019-01232-47.
7. Tucker K, Noe AR, Kotraiah V, Phares TW, Tsuji M, Nardin EH, *et al.* Pre-erythrocytic vaccine candidates in malaria. *IntechOpen* 2016; Chapter 16: 335-385.
8. Nino-Fong R, Jhonson JB. Poxvirus-based vaccine platforms: getting at those hard-to-reach places. *Future Virol* 2008;3(2):99-103.
9. Verma R, Khanna P, Chawla S. Malaria vaccine can prevent millions of deaths in the world. *Hum Vaccin Immunother* 2013;9(6):1268-1271.
10. Douglas AD, Baldeviano GC, Lucas CM, Lugo-Roman LA, Crosnier C, Bartholdson SJ, *et al.* A PfRH5-based vaccine is efficacious against heterologous strain blood-stage *Plasmodium falciparum* infection in Aotus monkeys. *Cell Host Microbe* 2015;17(1):130-139.
11. Smith JD. The role of PfEMP1 adhesion domain classification in *Plasmodium falciparum* pathogenesis research. *Mol Biochem Parasitol* 2014; 195(2): 82-87.
12. Raj DK, Nixon CP, Nixon CE, Dvorin DJ, DiPetrillo CJ, Pond-Tor S, *et al.* Antibodies to PfSEA-1 block parasite egress from RBCs and protect against malaria infection. *Science* 2014; 344(6186):871-877.
13. Ramesh V, Khanna P, Chawla S. Malaria vaccine can prevent millions of deaths in the world. *Human Vaccines & Immunotherapeutics* 2013;9(6): 1268-1271.
14. Rahman K, Khan SH, Fahad S, Chang MX, Abbas A, Khan WU, *et al.* Nano-biotechnology: a new approach to treat and prevent malaria. *Int J Nanomedicine* 2019; 14: 1401-1410.
15. Greenwood BM. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 2015;386(9988):31-45.
16. Aliwa B. Kenya strengthens fight against malaria with new vaccine. www.path.org/articles/a-first-for-kenya/ Last updated: 13 September, 2019.
17. www.gatesfoundation.org/What-We-Do/Global-Health/Malaria © 1999-2019 Bill & Melinda Gates Foundation.
18. www.who.int/immunization/diseases/malaria/malaria_vaccine_implementation_programme/en/ Last updated: 19 September 2019.
19. World Malaria Report, World Health Organization, Geneva 2019. Q & A on the malaria vaccine implementation program (MVIP).