Spotlights on new publications

Sherif M Abaza

Medical Parasitology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

Corresponding Author: Sherif M. Abaza, **Tel.:** +20 1005243428, **E-mail:** smabaza@hotmail.com

Received: 12 August, 2024**; Accepted:** 21 August, 2024.

Print ISSN: 1687- 7942, **Online ISSN:** 2090 -2646, **Vol. 17, No. 2, August, 2024**

New vaccine candidates V

In July 2022, the WHO listed 89 malaria vaccines, and 77 clinical trials among which 52 were completed. The present compilation presents four reports on the implementation of three malaria vaccines; RTS,S/AS01, *Pf*SPZ, and *Pfs*48/45. The first (Mosquirix™), designed by Glasgow Smith Kline (GSK®), Brentford, UK, was developed using the hepatitis B virus surface antigen (HBsAg) as a carrier matrix for circumsporozoite protein (CSP). Its lyophilized injection was administered IM in 4 doses in which the first three doses were administered monthly starting from the 5th month of age, while the last dose was administered at 15–18 m. Mosquirix™ represents the only malaria vaccine that advanced to phase III clinical trial. The second (Sanaria®), developed by Rockville, MD, USA, is composed of either live radiated (*Pf*SPZ) or chemo-attenuated (*Pf*SPZ-CVac) sporozoites. While the three IV doses of *Pf*SPZ are injected on days 1, 8, and 29, those of *Pf*SPZ-CVac, injected on days 1, 29, and 57, are accompanied by weekly chloroquine (CQ) administration (two days prior to the 1st dose and extended to the week after the last vaccination). Both are undergoing evaluation for their safety and efficacy in malaria-naïve, and exposed children and adults. Notably, Mosquirix™, and Sanaria® vaccine candidates were designed to target the pre-erythrocytic sporozoite stage to prevent human infection. On the other hand, *Pfs*48/45, *P. falciparum* sexual-stage antigen, constitutes two candidate vaccines (R0.6C and ProC6C) that share the 6C domain of *Pfs*48/45 antigen. The first utilizes the glutamate-rich protein as a carrier, while the second has a short 36-amino acid circum-sporozoite protein sequence. Both candidates are administered in 3 IM injections, 4 weeks apart. Since they induce antibodies against sporozoites, and prevent their transmission, they are termed TBVax1, *i.e.*, transmission-blocking vaccine.

RTS,S/AS01

In the year 2012, WHO recommended deployment of seasonal malaria chemoprevention (SMC) for children living in regions with moderate to high seasonal malaria transmission (SMT) to control transmission of malignant malaria. The SMC consisted of monthly intake of sulphadoxine–pyrimethamine plus amodiaquine administered to young children (4-5 times) during the SMT peak until they reach the age of 5 or 10 y. Although this strategy was widely applied to 45 million children during the year 2021 with high effectiveness, *falciparum* malaria remains the primary cause of high morbidity and mortality of young children in several high SMT regions, e.g., the Sahel and sub-Saharan Africa. Later, a combination of SMC and seasonal dose of RTS,S/AS01 vaccine was investigated in a clinical trial conducted in two countries with moderate SMT, *i.e.*, Burkina Faso and Mali. The results revealed that combined strategy was more effective than either intervention given alone over the period of the clinical trial (3 y). It showed marked reduction of clinical and severe malignant malaria during the trial period, and was sustained until the target children reached the age of 5 years when SMC is no longer given. The present clinical trial aimed to investigate the preventive efficacy of combined SMC with two seasonal booster doses of RTS,S/AS01 in young children in Burkina Faso and Mali. The study question was "Did this combination sustain protection for further two years?" A secondary objective was to assess its efficacy to decrease the incidence of meningitis and female mortality, previously recorded during the phase 3 trial (RTS,S clinical trials partnership, 2015).

To achieve their objectives, **Alassane Dicko** and his colleagues from Mali, Burkina Faso, USA, UK, and Belgium conducted a randomized, controlled, double-blind phase III clinical trial. The present trial was conducted on the same Mali and Burkina Faso districts and recruited 6861 children; among them were 5098 children who had completed the previous trial (combined SMC plus one dose of RTS,S/AS01), when they were 5-17 m old. They were randomly assigned to three groups; SMC alone, RTS,S/AS01 plus placebo, combined SMC plus double seasonal RTS,S/AS01 doses. Recruited children continued to receive the assigned regimens until the age of 5 years. Over the trial period (5 years), the investigators compared the outcome of the vaccine regimens according to incidence of clinical malaria.

Results revealed that the incidence of clinical malaria was 313, 320, and 133/1000 in the SMC, RTS,S/AS01, and combined regimen, respectively. Children who received the combined regimen had the

 DOI: 10.21608/puj.2024.312001.1263

highest protectiveness and effect, superior to either SMC or RTS,S/AS01. The study also observed that sole administration of RTS,S/AS01 versus SMC has superiority (~12% difference), but it is not inferior because there was not more than 20% increase in the incidence of clinical malaria. These results were more or less similar to the previous trial (3 y period) suggesting that single or double seasonal RTS,S/AS01 doses did not significantly improve the protective efficacy. Besides, hospital admissions for severe malaria, blood transfusion, and malaria mortality were reduced. The study concluded that substantial protection against *falciparum* malaria was sustained over 5 years by monthly SMC, and double seasonal RTS,S/AS01 doses. This approach offers a potential new strategy to control malaria transmission in regions with SMT. Compiled from **"Seasonal vaccination with RTS,S/AS01E vaccine with or without seasonal malaria chemoprevention in children up to the age of 5 years in Burkina Faso and Mali: A double-blind, randomized, controlled, phase 3 trial." Lancet Infect Dis 2024 Jan; 24(1):75-86.**

***Pf*SPZ**

Previously, two recent reports (Mwakingwe-Omari, *et al*. Nature 2021; 595(7866):289–294; Sulyok, *et al*. Nat Commun 2021; 12(1):2518) documented partial potential efficacy of chemo-attenuated *P. falciparum* sporozoites (*Pf*SPZ-CVac) accompanied by weekly CQ administration. Both studies showed that this vaccination approach induced dose-dependent protection (33-100%) against *falciparum* malaria. Vaccination was associated with a significant increase in polyfunctional memory $CD4^+$ T cells as well as circulating γδ T cells. The latter, a relatively small subset of T cells, are expressed by heterodimeric T-cell receptors (TCRs). Their composition of γ and δ chains, makes them different from the classical helper ($CD4^+$), and cytotoxic (CD^+) T cells. In the present compilation, Yoanne D Mouwenda *et al*., utilized mass cytometry to explore the immune profiles and mechanism(s) required for better protection. They compared the immune cells composition in malaria-naive European volunteers who received *Pf*SPZ-CVac plus single CQ dose per week with individuals living in areas under programs to control malaria, *i.e.*, with developed malaria immunity.

Results revealed that the response of CD4+ and γδ T cells, associated with protection in vaccinated volunteers, was similar to their role observed in malaria immunity. However, the investigators observed that the increase of γδ T cells was associated with higher induction of CD56 with its significant role in increased protection. In addition, protection was associated with expression of clusters of EMRA CD8+ and CD56+ T cells. The EMRA CD8+ are differentiated effector memory cells that express CD45RA, a marker usually found on naïve T cells. Besides, it is suggested that EMRA cells are derived from antigen-specific cells re-expressing CD45RA, indicating highly functional memory $CD8^+$ T cells. In comparison to placebo group, EMRA $CD8^+$ T cell clusters positive for CD38, and HLA-DR were significantly depleted in the vaccinated volunteers 8-10 w after the last inoculation. These clusters were previously recorded at day 22 after vaccination with *P. falciparum* circum-sporozoite protein, and apical membrane antigen-1. Therefore, the investigators attributed the significant depletion of CD38, and HLA-DR in their study to the long time it takes for migration to lymph nodes. The investigators concluded that upon vaccination, $CD4^+$, γδ, and EMRA $CD8^+$ T cells produced significant production of IFN-γ and TNF, with an immediate response eliminating *P. falciparum*.

Unfortunately, the study claimed three limitations: small sample size, absence of baseline, and lack of data regarding the specific *P. falciparum* antigens that drive host immune response. Accordingly, the study recommended future studies with a larger sample size to understand the dynamic mechanism of TCR clonotype that might help understanding the specific kinetics of EMRA $CD8^+$ T cells in response to malaria infections. Compiled from **"Immune responses associated with protection induced by chemoattenuated *Pf*SPZ vaccine in malaria-naive Europeans." JCI Insight 2024 May 8; 9(9):e170210.**

***Pf*s48/45**

In April 2024, there were two reports representing the first-in human phase I clinical trial that utilized *Pf*s48/45 vaccine to block malaria transmission.

Compilation of report no. (1)

In fact, *Pf*s48/45 antigen, expressed on the gametocytes' surface during development, is essential for fertilization, *i.e.*, male gametes lacking *Pf*s48/45 are not able to bind to female gametes in the mosquito midgut. When expressed in the human host, it remains hidden in RBCs, *i.e.*, cannot be targeted by antibodies. In the mosquito midgut, *Pf*s48/45 becomes accessible to antibodies present in the blood meal that prevent oocyst formation, and ultimately sporozoite development. Previous reports showed that immunization with either candidate sharing the 6C domain of *Pf*s48/45 antigen (R0.6C or ProC6C) elicited functional antibodies against sporozoites. In the present compilation, **Alfred B Tiono** and his colleagues developed a formulation, termed TBVax1, in which either candidate was adsorbed on Alhydrogel (AlOH) to enhance its stability, and uptake by antigen-presenting cells, as well. Since Matrix-M™ adjuvant accelerates production of high levels antibodies in humans, it was added to further enhance vaccine efficacy. The investigators conducted the first randomized, double-blind phase I clinical trial evaluating the potential efficacy of 30 µg or 100 µg R0.6C or ProC6C either adsorbed to AlOH alone or in combination with 15 µg or 50 µg Matrix-M™. The clinical trial was conducted on 125 healthy adults living in a malaria-endemic area of Burkina Faso.

Results revealed that both transmission blocking vaccine candidates were safe and well tolerated and induced IgG specific antibodies against the respective vaccine antigen. Only seven mild to moderate adverse events were recorded. The highest production of specific antibodies was recorded in volunteers immunized with 100 µg ProC6C-AlOH with Matrix-M™. Among them, 13/20 (65%) showed greater than 80% transmission-reducing activity (TRA). It is worth mentioning that immune-stimulatory activity of Matrix-M™ was significantly higher in humans than previously observed in mice. Moreover, combined Matrix-M™ and AlOH addition increased specific antibodies production 4.1-fold, and 6.2-fold in comparison with volunteers receiving 100 µg R0.6C and ProC6C and AlOH alone, respectively. The investigators explained the immune-stimulating activity of Matrix-M™ as due to recruitment of immune cells (e.g., dendritic cells, monocytes, and neutrophils) to the injection site facilitating drainages of antigen and immune cells to lymph nodes. Meanwhile, AlOH, serving as a vehicle, enhanced Matrix-M™ immune-potentiating effects.

In comparison to their respective controls, specific antibody titers were higher in volunteers receiving 100 µg ProC6C- or R0.6C-AlOH with Matrix-M /MM, measured at six months after vaccination. However, R0.6C induced sporadic reduction in malaria transmission. The investigators recommended further clinical trials investigating combination of both candidates to simultaneously reduce infection risks, and onward transmission to other individuals via mosquitoes. Compiled from **"A randomized first-in-human phase I trial of differentially adjuvanted *Pfs*48/45 malaria vaccines in Burkinabé adults." J Clin Invest 2024 Apr 1; 134(7):e175707.**

Compilation of report no. (2)

In the present open-label clinical trial, **Mark Alkema** and his colleagues from Netherlands, Sweden, and Denmark evaluated the safety, tolerability, and TRA of R0.6C in humans. The clinical trial recruited 31 malaria-naïve Dutch adults (18-55 y) who received four IM R0.6C doses (30 µg or 100 µg) on days 0, 28, 56 and 168. They were randomized for the allocation of one of two adjuvants, AlOH with and without Matrix-M™ adjuvant. Follow up began on the second day of injection and continued for 12 w after the last vaccination dose. Adverse side effects were recorded, anti-R0.6C, and anti-6C IgG antibodies were measured using ELISA. The TRA was determined by mosquito feeding assays using cultured *P. falciparum* gametocytes and laboratory-reared *A. stephensi* in the presence or absence of IgG antibodies. The TRA% was determined by the reduction of oocyst count in mosquitoes fed on gametocytes containing IgG antibodies compared to a non-serum control.

Results revealed that R0.6C was safe, immunogenic, and well-tolerated, with only one adverse event (transient fever). Specific IgG titres were similar in both doses (30 and 100 µg); however, they showed significant higher production in volunteers who received Matrix-M1™ adjuvant. In mosquito feeding experiments, sera collected from participants did not induce significant TRA; however, specific IgGs purified from sera collected 2 w after the last vaccination dose achieved up to 99% TRA. Accordingly, the study concluded that R0.6C induced insufficient serum antibody titres that achieve the threshold for reduction of malaria transmission in malaria naïve individuals.

From this report, two interesting observations were recorded. First, specific IgG antibodies against 6C fragment were only 10–40% of the total IgG, indicating that the greatest fraction of induced antibodies target the non-functional R0 fragment. Second, specific IgG response was not dependent on the vaccine dose (30 µg or 100 µg). Notably, the investigators also claimed two limitations: small sample size, and it was conducted during the SARS-CoV-2 pandemic; *i.e.*, there were limitations of follow-up visits outside the clinical trial protocols.

The investigators recommended future studies to identify alternative R0.RC formulations or regimens that enhance functional antibody responses for efficient TRA%. They also recommended evaluating the potential efficacy of R0.6C vaccine in endemic areas with moderate to high seasonal transmission to demonstrate superior antibody induction due to boosting of naturally acquired antibodies. Compiled from **"A *Pfs*48/45-based vaccine to block *Plasmodium falciparum* transmission: Phase 1, open-label, clinical trial." BMC Med 2024 Apr 23; 22(1):170.**