Spotlights on new publications

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New vaccine candidates VI

The present compilation presents five reports on the implementation of four malaria vaccines; RTS,S/AS01, *Pf*SPZ, RH5.1-Matrix-M, and ProC6C. As previously described [PUJ 2024; 17(2): 141-143], RTS,S/AS01 (Mosquirix™), designed using HBsAg and *P. falciparum* circumsporozoite protein (*Pf*CSP), is given IM in 4 doses (monthly three doses starting from the 5th month of age, and a fourth dose at 15–18 months). Sanaria® is composed of *P. falciparum* sporozoites (*Pf*SPZ) either live radiated or chemo-attenuated. Live radiated *Pf*SPZ vaccine is administrated in three IV doses on days 1, 8, and 29, while those of chemo-attenuated vaccine are on days 1, 29, and 57, and are accompanied by weekly chloroquine administration. In RH5.1-Matrix-M vaccine, manufactured by Clinical Biomanufacturing Facility in Oxford, UK, RH5.1 is mixed with 50 µg Matrix-M, a potent saponin-based adjuvant manufactured by Novavax™ (Uppsala, Sweden). Notably, RH5.1, a *P. falciparum* reticulocyte-binding protein homologue, is the most advanced blood-stage vaccine candidate. This highly conserved protein plays an essential role for RBCs invasion, On the other hand, ProC6C which is a recent multi-stage vaccine that includes *Pf*CSP targeting invasion of sporozoites to hepatocytes, is combined with *Pf*s48/45, and *Pf*s230 sequences targeting developmental stages in mosquitos. Therefore, ProC6C provides protection against primary infections, and reduces transmission of malaria from infected patients to susceptible risky individuals, *i.e.*, reducing the number of secondary infections. The 1st two vaccines (Mosquirix™, and Sanaria®) were designed to target the pre-erythrocytic sporozoite stage to prevent human infection. The 3rd vaccine is designed to target erythrocytic stages to lessen malaria severity, and prevent patient mortality and morbidity. The 4th is a multi-stage vaccine that inhibits infection in both human and mosquito populations. Notably, Mosquirix™ is the only malaria vaccine that advanced to phase III clinical trial.

***Pf*SPZ**

Since the majority of malaria vaccines that reach clinical trials aim to inhibit liver-stage development, *i.e.*, sporozoite entry into hepatocytes, attenuated or radiation-attenuated cryopreserved *Pf*SPZ vaccine was developed. However, its IV administration was less immunogenic, and less effective in trials for malaria-exposed African adults in comparison to malaria-*naive* adults. Sub-Saharan African infants, with low relative malaria exposure, showed the greatest risk for severe malaria and high mortality rates. Accordingly, it was hypothesized that prior low malaria exposure may enhance vaccine efficacy. Besides, a randomized placebo-controlled trial, conducted for Kenyan infants, showed that different dose administrations had non-significant results of *Pf*SPZ vaccine efficacy against parasitemia. However, infants who received the highest dose of radiation-attenuated *Pf*SPZ (1.8×10^6) exhibited the highest vaccine efficacy. These results suggested that the vaccine generated strong CSP-specific antibodies that modestly correlated with low or undetectable specific T cell responses protection. This relatively balanced immune response outcomes encouraged American and Kenyan investigators (**Leetah Senkpeil *et al.***) to investigate the molecular differences in-between vaccinated infants, *i.e.*, the underlying mechanisms for differential vaccine' immunogenicity and efficacy (protection against parasitemia). Utilizing artificial intelligence (machine learning) for pre- and post-vaccination blood samples, the investigators conducted a comprehensive analysis integrating clinical data, and whole-blood transcriptomic profile including CSP-specific antibody response, immunophenotyping, and plasma cytokines.

Results revealed that innate immune activation, and myeloid signatures at baseline (prior to vaccination) correlated with the protection level against parasitemia in placebo controls. Similar results were obtained among infants who received the highest vaccine dose. Spliceosome, proteosome, and resting dendritic cells signatures showed predictive features of protection after highest *Pf*SPZ dose, while baseline CSP-specific IgG predicted no protection against parasitemia. In contrast, pre-vaccination innate inflammatory, and myeloid signatures were associated with higher sporozoite-specific IgG antibodies, but with undetectable *Pf*SPZ-specific $CD8^+$ T cell responses post-vaccination. Consistent with these human results, *in vivo* innate stimulation exhibited protection against infection by sporozoite injection in malaria-*naive* mice with reduced $CD8^+$ T cell response to radiation-attenuated sporozoites. The investigators hypothesized

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that pre-vaccination baseline CSP-specific IgG was consistent with antibody feedback, in which recall responses against immunodominant epitopes are inhibited by pre-existing antibody response. Although there was reduction of specific cellular responses in some infants, a differential enrichment of T cell modules after *Pf*SPZ highest dose and associated protection against parasitemia was recorded. The obtained results from the systemic analysis suggested existence of a dichotomous role of innate stimulation to induce protective immunity, and protection against parasitemia. Additionally, the current compilation provided evidence for alternative mechanism(s) involving innate activation of myeloid cells. Analysis showed that while innate immune activation induced short-term protection against natural infection, it may prevent the liver-stage infection necessary for the vaccine to generate effective $CD8^+$ T cell responses, *i.e.*, induce durable protection against parasitemia. Besides, innate activation observed in peripheral blood reflected systemic activation affecting phagocytic tissue-resident macrophages, *e.g.*, Kupffer cells, the sporozoites portal into hepatocytes.

According to the obtained results, vaccine-induced protection was influenced by host factors, either intrinsic (age, and genetics), or extrinsic (preexisting immunity). Since differential malaria exposure between individuals remained a confounder, the investigators claimed that they adjusted their comprehensive systemic machine learning analyses on similar data for studied site, parasitemia, and preexisting CSP-specific IgG. However, they failed to adjust data related to coinfections, and intestinal microbiota that may contribute in the identification of specific microbial triggers of innate immunity. Accordingly, it was hypothesized that the protective efficacy of *Pf*SPZ in malaria-endemic areas may be controlled by contrasting antigen presentation pathways. Compiled from **"Innate immune activation restricts priming and protective efficacy of the radiation-attenuated *Pf*SPZ malaria vaccine." JCI Insight 2024 Apr 30; 9(11):e167408.**

RH5.1

The present compilation presents utilization of RH5.1-Matrix-M vaccine, a soluble protein vaccine candidate for *P. falciparum* erythrocytic stages. A previous study reported its safety and tolerability to induce cross-strain functional antibodies in malaria-*naive* British adults. In comparison to UK adults, another study showed that it induced ten-times higher antibody responses in Tanzanian infants (6-11 m). Accordingly, **Sarah E Silk** and her colleagues conducted a non-randomized clinical trial (phase Ib), first-in-human in Bagamoyo, a lower-transmission area in Tanzania. The clinical trial recruited healthy adults (18-45 y) and children (5-17 m) to administer three doses of RH5.1 formulated with 50 µg Matrix-M adjuvant. To evaluate vaccine' dose-escalation, the investigators designed their study using a dose escalation regimen in which participants received a delayed-fractional third dose regimen (DFx). Divided into two groups, adults were administered either 10 µg at 0, 1, and 2 m (10 µg monthly regimen, 10M), or 50 µg at 0 and 1 m, with a 3rd dose of 10 µg at 6 m (DFx). Similarly, children received 10 µg either at 0, 1 and 2 m (10M), or at 0, 1, and 6 m (10D). Additionally, two other groups of children were recruited to administer the dose escalation regimen. While the 1st group (DFx) was included to assess vaccine' dose-escalation, the 2nd group was recruited to measure the potential effect(s) of previous malaria exposure on RH5.1/Matrix-M immunogenicity. These children were recruited from a higher malaria pre-exposure area (HDFx). To select the last group, a prior study was conducted to determine IgG activity against *P. falciparum* schizont lysate using sera from children living in Bagamoyo' urban and rural areas. Low and high levels of antibodies of malaria pre-exposure were determined. Accordingly, IgG thresholds were used to assign children to HDFx group.

During four months (January-April, 2021), 12 adults, and 48 children, equally divided in 2, and 4 groups, respectively, were vaccinated. During the trial period (22 m after the last dose), the investigators recorded any adverse event after each dose to asses vaccine safety. After each dose, a home visit was done only on day 1 for all participants. On days 3, 4, 5, and 6 (for children), and 2, 7, 14, and 28 (for adults), participants were seen at the clinical research facility. For follow up, participants attended the facility on the 12th, 16th week, and after 10, 22 m. For determination of baseline and follow-up of CBC, SGPT, and creatinine levels (safety parameters), blood samples were collected on day 0, and at each follow up. To assess vaccine tolerability, adverse side effects were recorded during the first seven days after each dose, and during the clinical trial period. To evaluate vaccine immunogenicity, *i.e.*, magnitude, quality, and longevity of humoral and cellular immune responses to the vaccine, the investigators measured the concentration and avidity of anti-RH5.1 IgG antibodies, and their growth inhibition activity% (GIA) *in vitro*. Besides, RH5.1 cellular immunogenicity was determined using *ex vivo* ELISpot assay or flow cytometry. It is worth mentioning that the clinical trial also analyzed the frequency of RH5-specific plasma cells in the bone marrow of the vaccinated participants to assess the potential effect(s) of relatively higher malaria pre-exposure on vaccine-induced immune responses.

It was reported that 57 out of 60 participants (95%) completed the 3 vaccine doses, among them, 55 (92%) completed the trial period. The clinical trial claimed RH5.1 vaccine safety, and recorded no events related to vaccine administrations. Serum IgG, and specific T cell responses were induced by the vaccine. While purified total IgG showed *in vitro* GIA against *P. falciparum*, similar functional quality results were recorded, *i.e.*, GIA% per µg RH5-specific IgG. Two weeks after the last dose, the investigators showed that the concentration of RH5.1-specific polyclonal IgG required to give 50% GIA

was 14.3 µg/ml. Almost all 10D children (11 out of 12) showed the highest median anti-RH5 IgG concentration 2 w after the last dose. The obtained results confirmed that RH5.1-Matrix-M vaccine was well tolerated with acceptable safety profile using different doses, and regimens. However, DFx participants, whether adults or children, showed the highest anti-RH5 serum IgG responses following the 3rd dose. Additionally, HDFx children (with high malaria exposure) showed no apparent potential effects neither in functional GIA%, nor vaccine' immunogenicity. The investigators claimed that the trial had three limitations; small number of participants, lacking a control group, and using only *P. falciparum* 3D7 clone to determine GIA%. Therefore, they recommended further studies to accurately assess the functional antibody potency against different laboratory, and field *P. falciparum* isolates. Compiled from **"Blood-stage malaria vaccine candidate RH5.1/Matrix-M in healthy Tanzanian adults and children: An open-label, non-randomized, first-in-human, single-center, phase 1b trial." Lancet Infect Dis 2024 Oct; 24(10):1105-1117.**

ProC6C

Another approach for preventing malaria is passive transfer of highly potent human monoclonal antibodies (mAbs) against *Pf*CSP. Single administration of human *Pf*CSP-specific mAb induced high-level of malaria prevention for up to six months. For both active and passive immunization, it is important to precisely identify the *Pf*CSP epitopes that mediate protection after mAbs binding *in vivo*. Previous studies investigating the efficacy of RTS,S vaccine showed that the majority of inhibitory anti-*Pf*CSP polyclonal antibodies, and mAbs recognized NANP major repeat epitopes in the central repeat domain of *Pf*CSP. In contrast, a study that assessed *Pf*SPZ vaccine efficacy showed that some neutralizing mAbs recognized the NVDP epitopes motifs associated with minor repeats located immediately after the junction region of the N-terminus and *Pf*CSP domain. Notably, NANP and NVDP are important targets of antibodies isolated from people with naturally-acquired immunity to malaria. Accordingly, a novel malaria vaccine (ProC6C) was developed consisting of *Pf*CSP, the immune dominant antigen on the sporozoite surface which essentially contributes in hepatocytes invasion, and sequences of two *P. falciparum* antigens (*Pf*s48/45, and *Pf*s230). A short *Pf*CSP sequence composed of 6 and 3 NANP and NVDP copies, respectively was inserted between a structured domain (6C) of *Pf*s48/45, and pro-domain from *Pf*s230. Both domains are two transmission-blocking vaccine candidates. In fact, reducing malaria transmission is an essential step for malaria elimination.

In a previous phase I clinical, ProC6C adjuvanted with alhydrogel (AlOH) with and without Matrix-M™ was evaluated in Burkina Faso, and Mali. The vaccine proved safe and well tolerated, and it was reported that ProC6C-AlOH/Matrix-M was superior in immunogenicity to the vaccine immunogen (ProC6C). Besides, it induced significant transmission reducing activity (>80%) in 65% (13/20) of the participants that correlated to the Pfs48/45 antibody levels. In the present compilation (**Jordan Plieskatt *et al*.**) conducted a phase I clinical trial to describe the magnitude, specificity, avidity, and functionality of antibody responses on administration of ProC6C vaccine to recruiting volunteers from Burkina Faso. The vaccine was intramuscularly administrated (100 µg *Pf*CSP) either with AlOH alone obtained from Statens Serum Institute, Denmark, or combined with 50 µg Matrix-M adjuvant (ProC6C-AlOH/Matrix-M) obtained from Novavax©, Sweden. The study recruited 40 volunteers (20 for each regimen), and another 20 control who received Hepatitis B vaccine (Euvax B) obtained from LG Chem, Korea. Three doses were given on days 0, 28, 56, and sera were collected on days 0, 14, 28, 42, 56, 70, 140 and 180. Using antibody equivalence to *Pf*CSP mAbs (311, 317 and L9), the investigators determined mABs levels against full-length *Pf*CSP, and major/minor repeat peptides. A bio-layer interferometry was utilized to determine the antibody avidity. To determine antibody functionality, the human IgG was subsequently purified, pooled, and evaluated in a mouse sporozoite challenge model.

Results revealed that administration of ProC6C-AlOH/Matrix-M significantly enhanced antibody titers to major and minor repeats in all administered volunteers. After ten weeks, specific antiboes were more or less similar to levels obtained in Thai adults who received RTS,S/AS01 vaccine. Besides, ProC6C antibodies were found to be competitive to mAb 317, and mAb L9. Purified and pooled IgG, used in mouse sporozoite challenge model, showed a significant transmission reducing activity. In contrast, pooled IgG from controls, and pools of IgG with a low *Pf*CSP IgG concentration as well had no such activity, confirming that the protective effect was vaccine-specific, and dose-dependent.

It was concluded that ProC6C-AlOH/Matrix-M was an efficient multi-stage vaccine that successfully combined anti-infection and transmission-blocking efficacy. It had a significant impact in the reduction of malaria burden through reducing *P. falciparum* infection in both human and mosquito populations. The investigators claimed that a further study that will evaluate the standard three-dose monthly regimen of ProC6C-AlOH/Matrix-M in adult population in Mali is in progress to confirm its efficacy against malaria. Compiled from **"ProC6C, a novel multi-stage malaria vaccine, elicits functional antibodies against the minor and central repeats of the circumsporozoite protein in human adults." Front Immunol 2024 Nov 1; 15:1481829.**

RTS,S/AS01

Compilation of report No. (1)

Several clinical trials (phase III) were conducted to evaluate RTS,S vaccine efficacy in infants and children residing in several African countries with moderate-to-high *P. falciparum* transmission. The obtained results

revealed moderate reduction in malaria episodes (~30%, and 50% in infants and children, respectively) over a year that diminishes within 18 m. Due to declining efficacy, a fourth booster dose was recommended after 18 m that induced 36% efficacy over 4 y. Although the vaccine lowered morbidity and mortality rates associated with malignant malaria, it was realized that there is an urgent need for novel strategy to eradicate malaria. This necessitates developing a new vaccine or improving the efficacy of the available vaccines to increase vaccine durability and minimize malaria transmission. To improve vaccine efficacy to induce durable protection, it is important to identify and validate predictors of protection, *i.e.*, immune biomarkers that show significant association with vaccine-mediated protection. In fact, few mechanisms of RTS,S-mediated protection against sporozoite challenge were identified, and no immune response mechanism was accepted as predictive marker for RTS,S-ASO1 protection across different clinical trials. Several immune biomarkers were hypothesized including total IgG against the major NANP repeats, IgG1 and IgG3 targeting *Pf*CSP C-terminal region and NANP repeats, and antibodies avidity for the *Pf*CSP C-terminal region and binding to Fcγ receptors (FcγRs). Additional biomarkers associated with protection against malaria following RTS,S vaccination included natural killer cell activation, antibody-dependent cell phagocytosis, and engagement of FcγRIIIa. Accordingly, validation of correlates of protection (immune biomarkers) would facilitate the design of next-generation malaria vaccines by providing precise measures to improve efficacy and durability of RTS,S-induced antibodies. Additionally, this validation can support modifications of dose and schedule, or equivalence testing of product generated by different manufacturers.

To investigate the protection-predicting capability of total immunoglobulins, and specific IgG subclass induced by the vaccine, American and Belgium investigators (**Rachel L Spreng *et al.***) measured total serum antibodies and subclass antibodies (IgG1 and IgG3) responses using biolayer interferometry, and the binding antibody multiplex assay, respectively. Samples (No.=130) were collected from participants in two previous clinical trials [MAL092 (NCT03162614), and MAL102 (NCT03824236)]. Only samples of participants who previously provided consent for future research use (94, and 36 participants enrolled in MAL092, and MAL102, respectively) were included in this analysis. Immune responses were compared between protecting and non-protecting vaccinees using univariate binomial logistic regression models. Analysis was performed in a blinded manner without revealing knowledge of the true protection status. Besides, a multivariate modeling of combined data from three clinical trials conducted in controlled human malaria infection (CHMI) studies was performed. These studies included MAL068 (NCT01366534), MAL071 (NCT01857869), and MAL092.

Blinded prediction analysis revealed detection of several antibody binding measures that influenced protection-predicting capability. Magnitude-avidity composite of total antibody level specific for *Pf*CSP, serum major NANP repeats and N-terminal junction of *Pf*CSP, and magnitude of IgG1 subclass specific to *Pf*CSP and NANP major repeats had good prediction accuracy. While univariate analysis showed a significant association between these measures and protection (vaccine efficacy), multivariate modeling identified the combination of IgG1-NANP binding magnitude plus serum PfCSP NANP and N-terminal junction. The total antibody binding magnitude-avidity composite was the best predictor of protection with 95% confidence interval.

The study claimed that identification of absolute correlation of protection from malignant malaria is a challenging task due to the complexity of epitopes and immunodominant regions expressed on *P. falciparum* sporozoite. Effector function of the immune system, and the history of prior infections or exposures also influence and correlate with vaccine efficacy. Compiled from **"Identification of RTS,S/AS01 vaccine-induced humoral biomarkers predictive of protection against controlled human malaria infection." JCI Insight 2024 Oct 8; 9(19):e178801.**

Compilation of report No. (2)

To understand the immunological mechanisms of RTS,S-mediated immunity, **Liriye Kurtovic** and her colleagues presented the first report describing the association between antibody responses and protection against naturally acquired malaria. First, the investigators performed a PubMed search for clinical trials conducted on RTS,S vaccination in children. Forty-two articles were identified, among them 35 reported antibody responses, 18 evaluated the association between antigen-specific IgG and vaccine efficacy, and 4 evaluated IgG subclasses or avidity. It was worth mentioning that none of these studies evaluated the association between antigen-specific IgA and protection against malaria, or presented a stratified analysis by sex. Therefore, the primary objective of the present compilation was to identify antibody response types (antigen-specific isotypes and functions) measured one month after the last dose (M3), and their association with protection against malaria during 1.5 y-follow up after vaccination. Secondary objectives included evaluation of IgA functional mechanism(s), and performance of stratified statistical analysis by sex.

To achieve their objective, the study evaluated the Ig isotypes and Fc-dependent functional activities of antibodies induced by the RTS,S vaccine. The investigators performed post hoc analysis on serum samples collected during a phase IIb clinical trial conducted in Mozambique in 2003. Notably the vaccine efficacy for the first clinical episode was 29.9% in the first six months. Since the vaccine was administrated monthly for a three-dose vaccination schedule, all

available samples collected from the vaccinated children one month after the last dose (M3; No.=737) were analyzed. For comparison, the study included a subset of samples collected before vaccination (M0; No.=50); as well as control samples collected on M0 and M3 representing 25, and 99 samples, respectively. These blood samples were obtained from a study conducted in Australia during the period from 2018 to 2023.

To understand the potential mechanism(s) of immunity, the investigators utilized *P. falciparum* 3D7 reference strain, and quantified induction of antibodies to *Pf*CSP. Antigen-specific IgG, IgM, and IgA were determined using ELISA, and antibodies ability to mediate functional activities, *e.g.*, fixing serum complement proteins (C1Q), and binding to IgG Fcγ receptors (FcγRI, FcγRIIa, and FcγRIII), were evaluated using established immunoassays. To evaluate IgA ability to interact with FcαRI, the investigators used specific antibodies to CSP (mAbs 317, and MGG4), previously derived from human participants vaccinated with RTS,S, and a live-attenuated sporozoite vaccine, respectively. Notably, these antibodies were previously reported to be expressed on human IgA1 and IgA2, and to have the ability to bind to the sporozoites surface, and the NANP repeat of *Pf*CSP as well.

Results revealed that functional IgG responses to the C-terminal region of *Pf*CSP were associated with a reduced risk of malaria with significant results for complement component C1q, FcγRIIa, and FcγRIII. This result indicated the ability of IgM and IgG to fix C1q activation of the classical complement pathway to enhance antibody-mediated inhibition of sporozoite motility or invasion leading to its lysis. Significant protection was also associated with IgA response to the central repeat and C-terminal of *Pf*CSP. It was demonstrated that IgA had the avidity to bind with FcαRI mediating opsonic phagocytosis. Machine-learning (ML) analytical methods suggested that IgA, complement fixation, and FcγRI binding were most predictive mechanisms of protection against malaria. Furthermore, the investigators claimed that sex is increasingly recognized as an important factor influencing the vaccine efficacy. Using ML analysis, the investigators recorded notable differences in the associations between antibody responses and protection against malaria in male and female participants, *i.e.*, protection was stronger for male than female participants. Only IgA responses were significantly associated with protection among female participants.

Finally, the investigators discussed the study limitations, 1) evaluation of only young children in one malaria-endemic country, 2) adults are not included that may confirm or deny sex difference regarding functional antibody responses, 3) analysis was confined on RTS,S administered with AS02 adjuvant, while currently it is given with AS01 that contains two adjuvants (QS21 and MPLA). In a previous trial conducted for African children, the investigators observed higher induction of antibody responses with AS01 than AS02.

In conclusion, functional antibody responses mediated by IgG and IgA were associated with protection against malaria in young children vaccinated with RTS,S. The study revealed new insights into the mechanisms of RTS,S immunity and the investigators recommended further studies to explore the underlying mechanism(s) explaining sex difference of the functional antibody responses. This may require identification of the expression and functions of Fc receptors, functions of immune cells, *e.g.*, neutrophils and monocytes, encoding of genes relevant for immune function (cytokine receptors) among boys and girls, which were poorly investigated in malaria-endemic populations. Compiled from **"Antibody mechanisms of protection against malaria in RTS,S-vaccinated children: A post-hoc serological analysis of phase 2 trial." Lancet Microbe 2024 Oct; 5(10):100898.**