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TITLE: GMP Production and Clinical Trial of a Self-Assembling Protein Nanoparticle and Toll-Like Receptor Liposomal MPL Adjuvanted Malaria Vaccine

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14. ABSTRACT: A novel vaccine FMP014, adjuvanted with the Army Liposomal Formulation containing QS-21 (ALFQ) was assessed in a first-in human trial for safety and immunogenicity. FMP014 is a self-assembling icosahedral nanoparticle displaying the α-TSR domain of PfCSP, (NANP)6 CSP repeats, as well as two additional universal CD4+ epitopes specific to lymphatic choriomeningitis virus and influenza A virus. The vaccine was formulated in ALFQ, a liposomal adjuvant containing anionic vesicles 50 nm-30 µm in size, composed of cholesterol, synthetic phospholipids containing dimyristoyl fatty acid groups, 3D-PHAD (a synthetic Monophosphoryl Lipid A analog) and the immune-stimulator QS-21 (a saponin derived from the bark of the Quillaja saponaria). The trial enrolled ten subjects. Five subjects assigned to the low dose group received 20 µg of antigen (FMP014) with 0.5 mL of ALFQ, which contains 100 µg 3D-PHAD and 50 µg of QS-21. Five subjects were assigned to the high dose groups received 40 µg of antigen (FMP014) and 1.0 mL of ALFQ, which contains 200 µg 3D-PHAD and 100 µg of QS-21. The trial included a 3-dose regimen with vaccinations at 0, 29, and 57 days. Solicited adverse events were collected for 7 days post each vaccination. Unsolicited adverse events and serious adverse events (SAEs) are being collected through the studies' final visit. Antibody responses were assessed via ELISA. Initial data collected up to day 71 revealed that the vaccine displayed acceptable safety profiles and was immunogenic. Local solicited adverse events were generally mild and resolved by 72 hours. Systemic solicited adverse events were generally mild or absent in the low dose group and generally moderate in the high dose group. There were no related SAEs. Lab abnormalities were generally mild. The protective efficacy of FMP014/ALFQ against a Plasmodium falciparum controlled human malaria infection (CHMI) will not be determined as Part B of WRAIR #2652 has not been executed, and currently there is no foreseeable pathway for completion of this portion of the study.

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None listed

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INTRODUCTION

Plasmodium has a complex life cycle, and the different stages of the parasite's life cycle elicit specific and often non-cross-reactive immune responses. When the mosquito vector feeds on the mammalian host, sporozoites are introduced into the surrounding tissue around the bite and travel to the bloodstream. Sporozoites can enter liver cells within minutes or can take up to several hours following transmission by mosquito bite. In liver cells, the parasites multiply asexually as exo-erythrocytic stage parasites for between two and ten days, depending on the *Plasmodium* species (5-7 days for *falciparum*). The infected hepatocytes then release thousands of merozoites to initiate the blood (erythrocytic) stage of infection. The erythrocytic stage is responsible for the clinical symptomatology of malaria infection. In the vertebrate host, *Plasmodium* sporozoites traverse to hepatocytes via a complex passage initiating at the dermis and traversing through cellular barriers in the skin and the liver sinusoid. Induction of immunity targeted to molecules involved in sporozoite motility and migration into hepatocytes may lead to nonproductive and/or reduced hepatocytic infection. The effect of blocking these processes would be to reduce the potential invasion and amplification of parasites in the liver and thus reduce or eliminate the parasitic load. Preventative vaccines aim to protect malaria-naïve hosts from *Plasmodium* infection and/or clinical disease after the bite of an infected mosquito. Candidate proteins used for preventative vaccines include those expressed on the sporozoite or the early hepatic stage of the Plasmodium parasite. Of note, the *Plasmodium* circumsporozoite protein (CSP) antigen is a well-researched target that is found on the surface of the transmitted sporozoite stage, and consists of a conserved N-terminal region, a central region containing NANP and NVDP repeats, and a polymorphic C-terminus. An effective vaccine against CSP could confer sterile immunity by preventing infection of the liver. Currently, the RTS,S vaccine is designed using the CSP antigen and is the only available preventative vaccine. The malaria antigen utilized in this vaccine contains the NANP repeat and the polymorphic C-terminal region from CSP in conjunction with a hepatitis B virus-like particle and the AS01 adjuvant. However, vaccination with RTS,S showed limited and short-lived protection in those tested with ~50% vaccine efficacy. In spite of the development of high titers against the CSP antigen, protection waned within a few months, and this coincided with the loss of protective antibodies. In addition, the immune responses and protective efficacy seem to be species-specific and/or strain-specific, with varied efficacy depending on the region. These results demonstrated that CSP is still a promising vaccine target, but the formulation and design require additional modifications to elicit optimal protection.

Experimental goals to increase potency, longevity and breadth of protection elicited by RTS,S warrant the development of similar vaccine designs with altered delivery mechanism, immunogenicity, or structural attributes of the vaccine candidate. Importantly, the RTS,S vaccine utilizes a liposomal adjuvant called AS01. Liposomes are small spherical vesicles formed from mixing phospholipids and cholesterol, which interact to form an outer phospholipid bilayer with an aqueous center. The use of liposomes as a method for vaccine delivery was recognized when it was demonstrated that these biocompatible molecules could selectively target immune cells 8. The

AS01 formulation includes a modified, non-toxic analog of lipopolysaccharide from Salmonella known as MPL and a saponin molecule derived from the tree bark of *Quillaja* species named QS-21. The Army Liposome Formulation (ALF) is an alternative liposomal adjuvant that also incorporates synthetic MPL. Multiple clinical trials and animal studies have evaluated ALF, including experiments evaluating side effects and protective immunity elicited with malaria, prostate cancer, and HIV antigens. Recently, addition of QS-21 to ALF (ALFQ) was tested in several studies, namely with malaria and HIV vaccine antigens, and was found to be well tolerated, effective at increasing antibody titers and cytokine responses, and elicited protective efficacy in animal models.

A novel vaccine was designed to express *Plasmodium falciparum* CSP named, *P. falciparum* Malaria Protein # 014 (FMP014) formulated with the WRAIR proprietary ALFQ adjuvant. Preclinical studies in animal models elicited potent immune responses to the pre-erythrocytic stage of Plasmodium, which resulted in impressive protective efficacy (Seth et al). As a follow-up to these studies, the objective of the current study was to evaluate the safety, immunogenicity, and protection conferred by this vaccine candidate in healthy, malaria-naïve adult subjects. Based on the success of the predecessors of the current ALF adjuvants formulations, as well as CSP-derived vaccines, and in addition to results so far investigating FMP014 formulated in ALFQ, the next phase of investigation was the evaluation for this vaccine candidate in first-in-human, Phase I clinical trial to determine the safety, immunogenicity and efficacy in Controlled Human Malaria Infection (CHMI). The information below is the preliminary assessment of the safety and immunogenicity results.

Vaccine Components Produced Under The Current Award:

FMP014 (Falciparum Malaria Protein #14)

- Self-Assembling Nanoparticle with highly repetitive, symmetrical CSP antigen (3D7 *P. fal*)
- Pre-erythrocytic antigen: partial C terminus, 6 NANP repeats, 2 CD4 epitopes specific to LCMV and influenza A virus
- Monomer expressed in/purified from *E. coli* manufactured at the WRAIR PBF
- Subsequent refolding techniques allow 60 monomers to "oligomerize" into 1 nanoparticle
- Found to be safe, well-tolerated, and immunogenic in C57Bl/6 and Balb/c mice

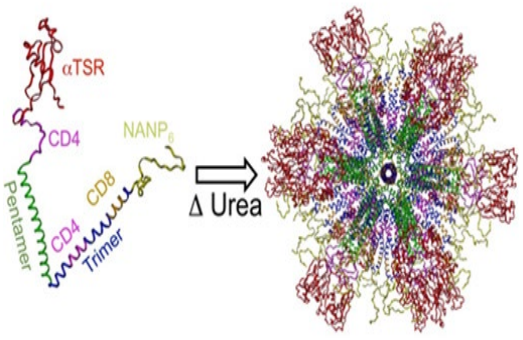


Figure 1: SAPN Nanoparticle Structure

ALFQ Adjuvant

- Liposomes with 3D-PHAD®, DMPC, DMPG, QS21, and 55% cholesterol
- Full dose has 4 times the 3D-PHAD® of the MPLA in AS01B
- Full dose has 2 times the QS21 in AS01B
- Found to be safe and well-tolerated in mice, rabbits, and rhesus macaques

Component		Human dose
ALF55 Lot#1974	3D-PHAD®	200 µg
	DMPC	13.972 mg
	DMPG	1.577 mg
	Cholesterol	5.413 mg
QS-21		100 µg

First in human (FIH) Phase I Safety and Immunogenicity Study in US naïve subjects

Primary:
- To assess the safety of candidate malaria vaccine FMP014/ALFQ

Secondary:
- To determine the protective efficacy of FMP014/ALFQ against a *Plasmodium falciparum* controlled human malaria infection (CHMI)
- To measure immune responses to CSP, induced by FMP014/ALFQ, using various immunoassays

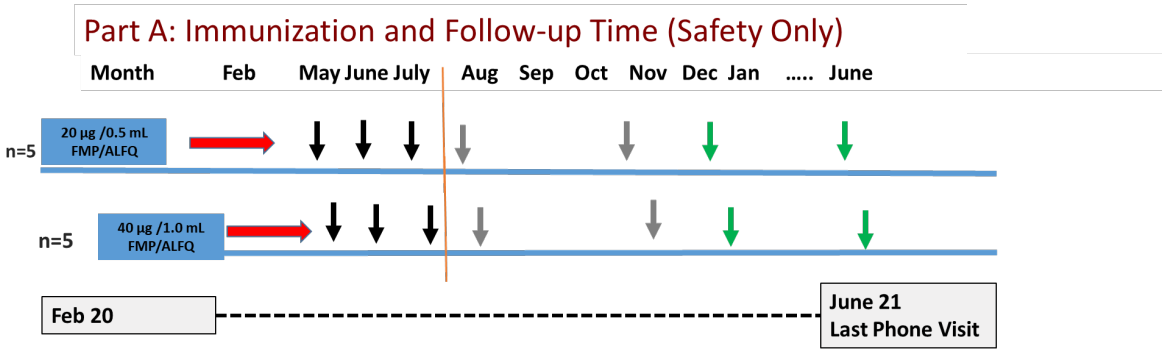
Exploratory:
- To compare the protective efficacy of standard, delayed dosing, and delayed fractional dosing against a *Plasmodium falciparum* controlled human malaria infection (CHMI)

Safety Assessment:
- Safety will be assessed by the incidence of adverse events (AEs), including all AEs, solicited AEs, unsolicited AEs, and serious for FMP014/ALFQ

Study design: This investigation was a Phase 1, open-label, single center study. The primary objective was to assess the safety and reactogenicity of FMP014/ALFQ. The secondary objective was to measure immunogenicity induced by the vaccine at pre-specified time points. The trial was conducted at the WRAIR Clinical Trials Center in Silver Spring, MD and approved by the WRAIR Institutional Review Board (IRB). Written informed consent was obtained prior to subject involvement in study activities. Study activities were conducted in accordance with all applicable Federal and Department of Defense human research protections requirements under FDA IND (Clinical Trials.gov identifier NCT04296279).

Three vaccinations were administered in a dose-escalation trial design utilizing sentinel participants with doses ranging from a 20 µg FMP014/0.5mL ALFQ (Low dose group) to 40 µg FMP014/1.0 mL ALFQ (High dose group) at study days 1, 29, and 57, with the last in-person visit on day 169. Follow up safety phone calls occurred on days 225 and 393 of the trial. Safety and humoral response data were collected throughout the study period.

Figure 2: Study Timeline - Part A Safety and Immunogenicity



STUDY METHODS

Studies Design and Participants

- Phase 1, open-label, single center, parallel design conducted at the WRAIR Clinical Trials Center (Silver Spring, MD) to assess a novel *P. falciparum* circumsporozoite vaccine candidate with the adjuvant (ALFQ) for safety and immunogenicity .
- Study reviewed/approved by WRAIR Institutional Review Board (FMP014: NCT04296279) and all participants provided written informed consent before enrollment
- Subjects were healthy, malaria naïve, non-pregnant adults aged 18-55yrs (inclusive) recruited from the Baltimore-Washington metropolitan area
- 10 subjects: 5 low dose (20 µg antigen/0.5ml ALFQ) per study, 5 high dose (40 µg antigen/0.5ml ALFQ); 1 sentinel subject per dose per study (4 total)
- Primary Objectives: To assess the safety and reactogenicity of candidate malaria vaccines
- Secondary Objectives: To measure CSP-specific immune responses induced by the

Study Procedures

Safety Assessment

- Occurrence of local injection site and solicited AEs from enrollment to final follow-up visit
- Occurrence of unsolicited AEs from enrollment to final follow-up visit
- Occurrence of serious adverse events (SAE), medically attended adverse events (MAAE) at any time during the study period (including 12 month post last vaccination f/u phone call)

Immunogenicity Assessment

- Measure antibody, innate, and cellular responses to PfCSP present in serum/blood at specified time-points
- Full Length CSP (presented), (NANP)6, Pf16 antibody titers and avidity assessed at baseline (day 1), days 29, 57, 71, and 169
- Cell mediated immunity (CMI) assays at days 57, 71 and 169
- Cytokine assays days 2, 30 and 58

Part A of WRAIR #2652 (First-in-Human Testing of FMP014/ALFQ) involved the enrollment of 10 subjects (5 high dose and 5 low dose) to complete a series of 3 vaccinations on a 0, 1, 2 month schedule [10/10 of the FMP014/ALFQ subjects]. By June 30, 2021, Part A of WRAIR #2652 had

completed the final day 393 phone visits [12 months after the 3rd vaccination (final receipt of Investigational Product for Part A participants.)]

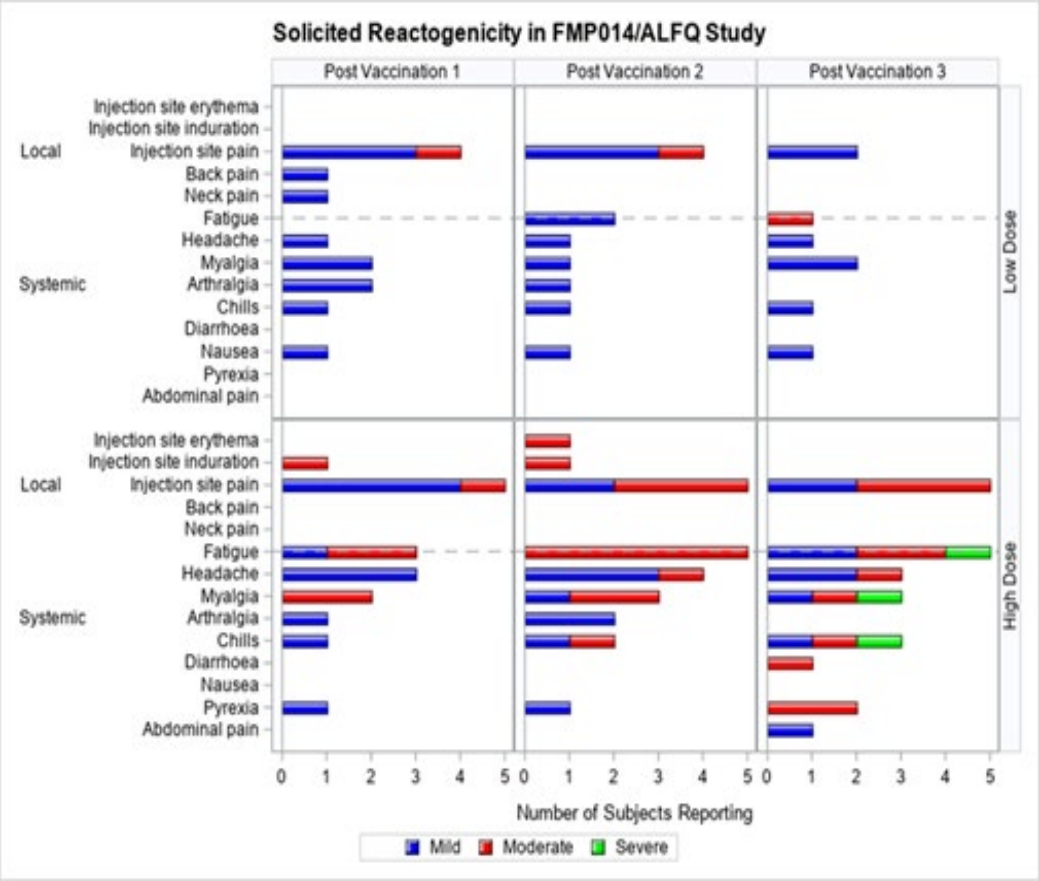
Safety and tolerability

Overall, 10/10 of the FMP014 subjects completed all three vaccinations. One high dose subject moved out of state shortly after vaccination #3 due to a family emergency. The vaccinations were generally well tolerated with increasing reactogenicity with the higher dose and subsequent vaccination. Mild local reactions were experienced in a majority of low dose volunteers for all three vaccinations, with a majority of high dose volunteers experiencing moderate local reactions after vaccinations #2 and #3. One high dose FMP014 subject had over a week of moderate redness, pain, and induration at the injection site after the 2nd vaccination, they had reported a similar reaction to one of the doses of the HPV vaccine many years prior. Only a minority of the subjects receiving the low dose of FMP014 experienced systemic adverse events after each vaccination, and they were almost universally reported as mild.

The FMP014 high dose group experienced more solicited systemic AEs, starting at vaccination #1 with mostly mild symptoms, increasing to mostly moderate systemic symptoms after vaccination #2, and peaking with one subject having less than 24 hours of severe flulike symptoms (severe chills, severe myalgia, severe fatigue, severe subjective fever with measured peak temperature of 101.5 ^{o}F, which is graded as moderate) after vaccination #3. Measured fevers were only seen in the high dose group and limited to a minority of that group. The only severe solicited AEs were the collection of the three flulike symptoms accompanied by a moderate measured fever mentioned above that occurred in one subject shortly after the 3rd dose lasting less than 24 hours. Figure 3 illustrates the trends in reactogenicity increasing with dosage number and strength of dose. No deaths or serious adverse events were reported. Most solicited AE's resolved by 72 hours post-vaccination. Lab abnormalities were mostly mild and lacked association with dosage or timing of vaccination. Unsolicited potentially related adverse events were rare and mild in grade. On the day 169 follow-up visits, AEs reported were *unlikely* or *not related* to the investigational product. Phone visits performed on days 225 and 393 revealed no MAAE's or pIMD's.

In summary, ALFQ local reactogenicity appears to be comprised primarily of local tenderness that is omnipresent, mild, and short-lived across both products and doses. Systemic reactogenicity primarily consists of a flu-like response that is also 24-36 hours in length and increases in intensity with dose: low-dose (mostly absent or mild), FMP014 high dose (mild to moderate severities after the first dose with mostly moderate severities after each dose with one subject having 24 hours of severe fever, chills, myalgias, and moderate temperature of 101.5˚F after the third dose).

Figure 3: Solicited Reactogenicity in FMP014/ALFQ Part A



Footnote: For low and high dose groups, all subjects received all three vaccinations. For multiple adverse events per subject per vaccination within a preferred AE term, only the highest severity grade are selected.

Immunogenicity assessments characterizing the immune responses to CSP induced by FMP014/ALFQ have included measurement of antibody titers and antibody avidity via ELISAs to full length PfCSP, peptides containing $(NANP)_6$ and a c-terminal peptide, Pf16. Assessment of antibody functional inhibition via *in vitro* inhibition of liver stage development assay (ILSDA), the opsonic phagocytic activity relative to CSP antibody titers, and the vaccine-induced cytokine profiles by MSD and fluorospot ELISpot methods have added to the body of knowledge regarding elicitation of anti-CSP immune responses.

Figure 4: Kinetics of Antibody Response to Full Length PfCSP
Immunogenicity was assessed at specified time pre- and post-vaccination time points by measuring innate, humoral, and cell-mediated responses.

ELISA: The three vaccinations were given on days 1, 29, and 57 and serological assays were performed on day: -7 (Pre-bleed), day 29 (4WP1), day 56 (4WP2), 71 (2WP3), and day 169 (16WP3). ELISA was performed essentially as described previously, and titer was defined as the

serum dilution that resulted in OD=1. To measure changes in the quality of the humoral response, an avidity ELISA was performed and avidity index was described as the percentage of antibodies that remained bound following urea wash. ELISA assays were performed against recombinant CSP-FL, CSP repeat $(NANP)_6$, or the C-terminal Pf16 peptide plate antigens.

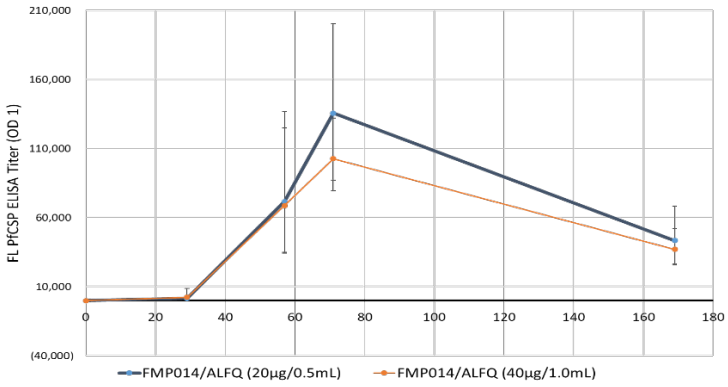
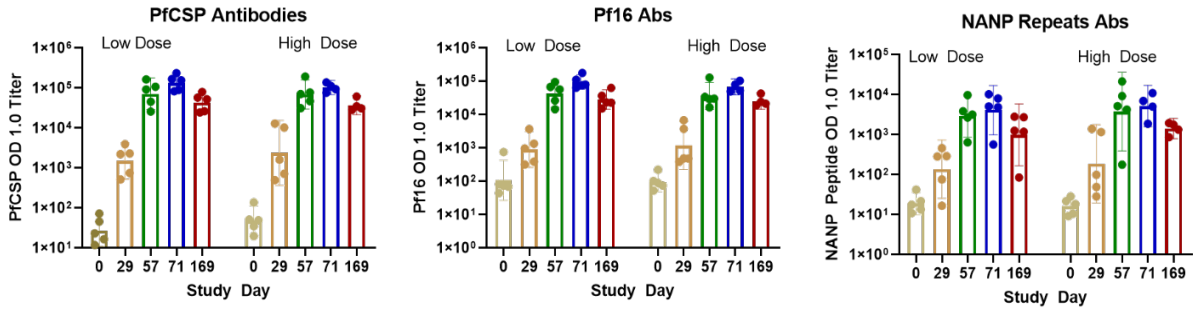


Figure 4: Kinetics of Antibody Responses for Low and High Dose Groups

The data are reported as the geometric means and 95% confidence intervals for responses to the full length PfCSP protein.

At all-time points tested, no apparent difference detected between antibody responses induced by low dose and high doses of FMP014/ALFQ. The kinetics of antibody decay showed an about three-fold reduction in antibody by the last study day.

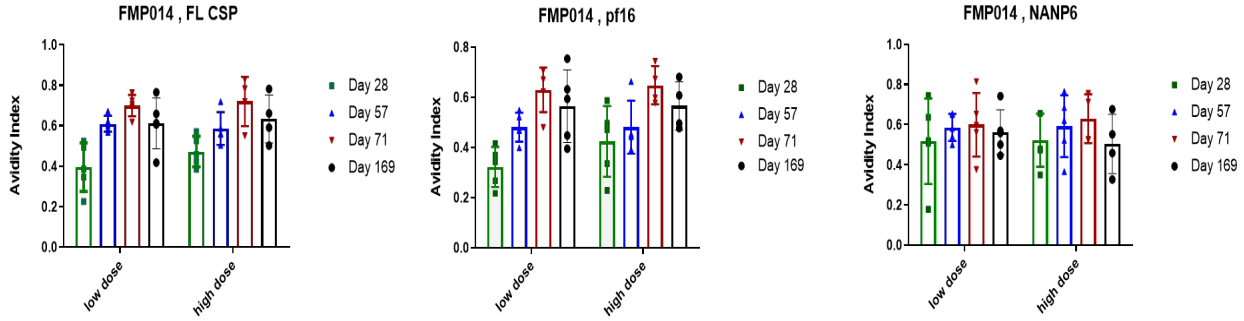
Figure 5: Antibody Responses to FLCSP, $NANP_6$ repeat and C-terminal peptide Pf16 (individual value plots shown to reveal the cluster of responses)



Kinetics of PfCSP, Pf16 and NANP antibody titers, individual value plots as bar graphs - Data are reported as the geometric means and 95% confidence intervals

The antibody reponses peak following the third dose for both low dose and high dose vaccine groups. No significant difference in antibody responses by plate antigen are detected for the two different vaccine dosage groups suggesting that the low dose is sufficient for inducing high titer antibody responses. The SAPN particle vaccine primes a significant response after a single dose, and the third dose does not significantly boost after the second dose suggesting that either the dose is too high and/or the interval is too short between the second and third dose. Additional analysis to address the impact of schedule cannot be extrapolated since the Part B was cancelled.

Figure 6: Kinetics of Antibody Avidities to CSP Subunits

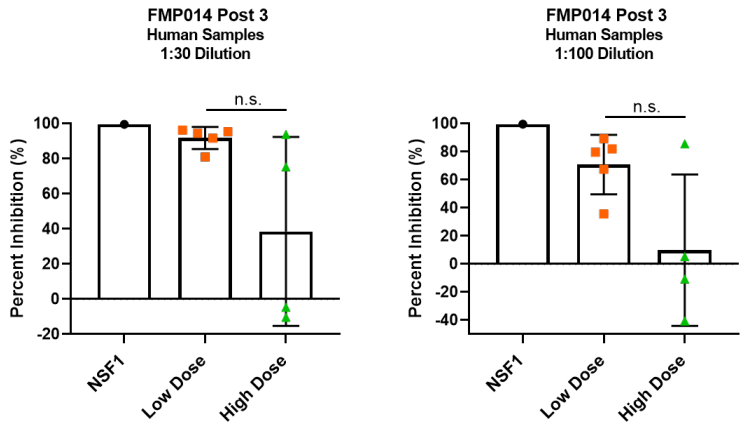


Kinetics of PfCSP, Pf16 and NANP avidity of antibody titers, individual value plots as bar graphs -
Data are reported as the geometric means and 95% confidence intervals

Generally, antibody avidities peak following the third dose for both low dose and high dose vaccine groups. Overlapping confidence intervals reveals that antibodies are relatively stable even up to study day 169, with no difference in avidity with the post 3rd responses. No significant difference in antibody avidities are detected by dosage group suggesting that the low dose is sufficient for inducing high quality antibody responses.

Figure 7: Functional Inhibitory Antibodies Measured in ILSDA

Inhibition of Liver Stage Development Assay (ILSDA): ILSDA was performed as described previously. Briefly, NF54 Pf sporozoites were incubated with sera at two weeks post third immunization (2WP3) from immunized individuals and their corresponding pre-bleeds at 1:30 and 1:100 dilution. Anti-CSP monoclonal NFS1 was used as a positive control. This mixture was then added to wells containing human hepatocytes and after incubation Pf 18S rRNA levels were determined by quantitative real-time PCR. Percent inhibition was calculated against the naïve serum negative control and the assay was repeated twice. Depending on the dilution, the low dose samples mean percent inhibition was >90% and >60% at 1:30 and 1:100, respectively. No statistical significant difference was seen between the low and high dose groups.



Percent inhibition of Post 3rd Serum Antibodies for Low dose and high dose groups Because of the spread in the high dose group, there was no significant difference at either dilution tested.

Briefly, serum samples at day 71 inhibited sporozoite invasion into human hepatocytes as measured by an ILSDA at 1:100 serum dilution **(Figure 7)**. Previous work on RTS,S has revealed an inverse relationship between opsonophagocytosis assay (OPA) index and protection *[Ref: Chaudhury S, Regules JA, Darko CA, Dutta S, Wallqvist A, Waters NC, et al. Delayed fractional dose regimen of the RTS,S/AS01 malaria vaccine candidate enhances an IgG4 response that inhibits serum opsonophagocytosis. Scientific reports. 2017;7:7998*]. The Day 71 sera tested positive on this OPA

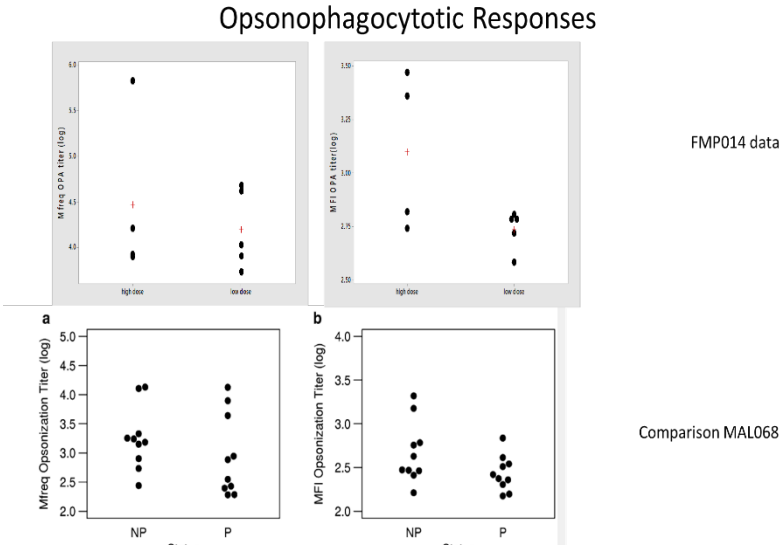
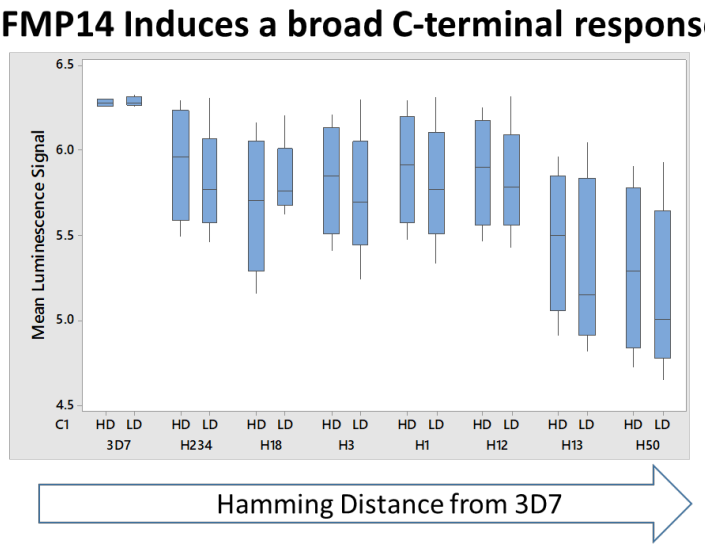


Figure 8: Opsonophagocytotic Responses

Opsonophagocytosis responses: The data are reported as log-transformed. The red cross mark is the mean response of the n=4 (high dose group) or n=5 (low dose group) volunteers. For reference is shown the results from the published MAL068 data → where the phagocytic activity is slightly higher than what was seen for RTS,S – where the lower titer was associated with protection.

Conclusion: From these results we conclude that the low dose of the FMP014/ALFQ yielded superior responses.

Figure 9: Breadth of Antibody Reponses to C-terminal Peptides to Assess Cross-Reactivity



Responses (expressed as Mean Luminescence signal) are plotted based on the Hamming distance from P. falciparum 3D7 parasite sequence. FMP014 responses are higher to the individual peptides than what was previously published for the malaria vaccine candidate, MAL27 RTS,S/AS01B (benchmarked).

Briefly, previous work demonstrated a positive correlation between wider C-terminal antibody breadth and protection using RTS-S-immune sera [Ref: *Chaudhury S, MacGill RS, Early AM, Bolton JS, King CR, Locke E, et al. Breadth of humoral immune responses to the C-terminus of the circumsporozoite protein is associated with protective efficacy induced by the RTS,S malaria vaccine. Vaccine. 2021;39:968-75*]. Day 71 sera tested for C-terminal cross-reactivity showed lower reactivity to heterologous peptides (H234, H18, H3, H1, H12, H13, H50) as compared to the homologous 3D7 strain peptide (Fig 8). A trend towards improved overall breadth of C-terminal region antibody responses was also observed for the High dose group.

Overall Conclusions from Part A Safety and Immunogenicity Study:
The objective of this study was to evaluate a novel malaria vaccine for safety and immunogenicity in healthy non-immune adults. This first-in-human study of both the FMP014 antigen and the ALFQ adjuvant demonstrated an acceptable safety and tolerability profile. FMP014/ALFQ elicited antibodies that bound to the NANP repeat and C-terminal peptides. Sera inhibited the invasion *P. falciparum* sporozoites and possessed positive opsonophagocytosis activity.

Status of Products Developed under this Award

FMP014 Drug Product: In February 2021, at the stability T 60 months' time point, FMP014, lot 1973 (drug product) failed testing on protein content by A280 measurement. A brief investigation ensued to address the issue an Out of Specification (OOS) and it was determined that the Lot 1973 Drug Product was no longer suitable for testing in clinical studies due to a downward shift in protein concentration. In June 2021, the attempted a refill of 500 new vials of the FMP014 Drug product, using Lot 1966 stored Drug substance. On thawing of the bulk material, visual observations showed visible particulates. Following dilution and filtration through a 0.2 micron

filter, particulates remained in suspension. This caused the refill and vialing of a new lot FMP014 to be terminated. Analysis of the diluted, filtered drug product (pre-vialing) indicated that the particulates were precipitated (unstable) protein materials. Additional testing using Dynamic Light scattering suggested that the pre-filtered protein material was highly heterogeneous suggesting that the produce was no longer stable. A final decision was made to terminate the refilling of the FMP014 drug product. This information was reported to MRDC ORA, and stakeholders/partners. No additional work has been done to address the issue of product stability.

ALFQ Adjuvant: Stability testing is ongoing; as of October 2022 achieved T79. ALFQ remains in active clinical evaluation with other vaccine antigens, such as the 1) WRAIR Biologics Research & Development (formerly Malaria Biologics Branch) FMP013 soluble *P. falciparum* CSP which completed the Part B, efficacy testing and 2) WRAIR EID's SpFN COVID-19 vaccine formulated in ALFQ which recently completed Phase I safety and immunogenicity assessment.

Part B of WRAIR #2652 has not been executed due to logistical constraints, and currently there is no foreseeable pathway for completion of this portion of the study. The initial logistical hurdle for starting Part B of the study in March 2021 was the high number of potential volunteers receiving COVID-19 vaccines at that time. Per the exclusion criteria volunteers cannot receive another vaccine within 14 days of the study IP (before or after). Based on the dosing schedule for the Pfizer and Moderna EUA vaccine products, this functionally creates a 56 day window during which potential volunteers are either ineligible for study enrollment, or must choose to defer receipt of COVID-19 vaccination. This led to challenges in recruiting volunteers. The parallel study with a planned 2 week earlier start (WRAIR #2651) was able to fill, but it was decided to delay Part B of this study until most potential volunteers had already completed the COVID-19 vaccination series.

While tentatively planning for an adjusted date for first Part B vaccine dose administration the vialed FMP014 investigational product failed to meet specifications during the May 2021 scheduled stability assessment. As preliminary analysis suggested the particulate matter visualized was aggregated FMP014 protein (not foreign material), we coordinated for the Pilot Bioproduction Facility to generate newly vialed samples of FMP014 from the -80° C stored bulk product. However, when this was attempted in July 2021 aggregation was noted in the thawed bulk product, and again during the inspection of the post-filtration diluted bulk drug substance, and any vials of drug product generated would not pass inspection for their release. As such, there is insufficient Investigational Product to conduct Part B of the study. We explored the concept of an entirely new bulk production run, but a comprehensive analysis of the relevant factors (cost and availability of needed resources; likelihood of success) argued against pursuing this course of action. The study team has been conducting the steps necessary to achieve final closure by Sponsor (i.e. database lock) and completion of all study related activities.

The findings from this study are currently being prepared as a manuscript.