



First-in-human assessment of safety and immunogenicity of low and high doses of *Plasmodium falciparum* malaria protein 013 (FMP013) administered intramuscularly with ALFQ adjuvant in healthy malaria-naïve adults

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ABSTRACT

The global burden of malaria remains substantial. Circumsporozoite protein (CSP) has been demonstrated to be an effective target antigen, however, improvements that offer more efficacious and more durable protection are still needed. In support of research and development of next-generation malaria vaccines, Walter Reed Army Institute of Research (WRAIR) has developed a CSP-based antigen (FMP013) and a novel adjuvant ALFQ (Army Liposome Formulation containing QS-21). We present a single center, open-label, dose-escalation Phase 1 clinical trial to evaluate the safety and immunogenicity of the FMP013/ALFQ malaria vaccine candidate. In this first-in-human evaluation of both the antigen and adjuvant, we enrolled ten subjects; five received 20 µg FMP013 / 0.5 mL ALFQ (Low dose group), and five received 40 µg FMP013 / 1.0 mL ALFQ (High dose group) on study days 1, 29, and 57. Adverse events and immune responses were assessed during the study period. The clinical safety profile was acceptable and there were no serious adverse events. Both groups exhibited robust humoral and cellular immunological responses, and compared favorably with historical responses reported for RTS,S/AS01. Based on a lower reactogenicity profile, the 20 µg FMP013 / 0.5 mL ALFQ (Low dose) was selected for follow-on efficacy testing by controlled human malaria infection (CHMI) with a separate cohort.

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Abbreviations: ALFQ, Army Liposome formulation containing QS-21; CHMI, controlled human malaria infection; CI, confidence interval; CSP, circumsporozoite protein; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunosorbent spot; GMP, good manufacturing process; AE, adverse event; SAE, Serious adverse event; ILSDA, inhibition of liver stage development assay; MAAE, medically attended adverse event; PIMD, potentially immune-mediated disease; WRAIR, Walter Reed Army Institute of Research; 4WP1, 2WP3, 4WP2, 16WP3, 4, 2 or 16 weeks post first, second or third vaccination; MSD, Meso Scale Diagnostics..

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1. Introduction

Malaria is a major global contributor to mortality and to diminished quality of life, with an estimated 241 million cases and 627,000 deaths in 2020 (World Malaria Report, Dec 2021). An effective malaria vaccine, in addition to other methods of malaria control could eliminate a large number of these deaths [1,2]. The immunodominant CSP coat of the *Plasmodium falciparum* sporozoites is comprised predominantly of the Circumsporozoite protein (CSP) and CSP-based vaccines can induce an immune response capable of protecting against malaria infection [3–6]. CSP consists of

an N-terminal ‘domain’ comprised of the inter-species conserved KLKQP motif or the Region I (RI), followed by the junctional sequence NP-DPNA-NPNV-DPNA. The central region of CSP contains 25–42 copies of NPNA, interspersed with NPNV and DPNA repeats [7–10]. The C-terminal region of CSP contains an alpha thrombospondin type-I repeat domain. *P. falciparum* CSP N-terminal and repeat regions show a high degree of conservation, while the C-terminal region harbors significant inter-strain polymorphic variability [11].

RTS,S is a particle-based antigen, containing the NPNA repeats and the C-terminal region of CSP fused to the hepatitis B surface antigen. RTS,S is co-formulated with the adjuvant AS01 that contains a Toll like receptor 4 (TLR4) agonist, Monophosphoryl Lipid A and the immune-stimulant QS21 [12]. In Phase 3 trials, a pediatric formulation of RTS,S showed 30–50% efficacy against naturally transmitted malaria, the efficacy was estimated at ~ 60% when combined with chemoprevention [1]. In October 2021, the WHO recommended RTS,S/AS01 vaccine (Mosquirix, GSK Biologicals) use to prevent *P. falciparum* malaria in children living in regions with moderate to high transmission. RTS,S/AS01's efficacy in the field is relatively modest and diminishes over time, with some concern that malaria cases could rebound as RTS,S immunity wanes [13]. While the WHO recommendation regarding RTS,S/AS01 is without question a monumental step forward, improvements beyond the performance of RTS,S/AS01 and other first generation vaccines are needed in the battle for malaria elimination. Beyond advancements in adjuvant formulation, optimization of dose and regimen to maximize vaccine efficacy [14,15] future efforts are aimed at iterative improvements in longevity [13], cost [16] and breadth of protection [17] elicited by CSP-based vaccines.

The Walter Reed Army Institute of Research (WRAIR) has sought to optimize the CSP antigen and the adjuvant. Leveraging decades of benchmarking experience, an optimized nearly full-length molecule, FMP013 (Falciparum Malaria Protein 013), was advanced to clinical evaluation. FMP013 is a soluble protein designed to contain the N-terminal region, the junctional repeats (3xNPNV and 3xDPNA) and 15 copies of the major NPNA repeats along with the C-terminal region of CSP [18]. The rationale being that although soluble CSP candidates were tested early in malaria vaccine development efforts, none were evaluated in concert with potent modern adjuvants such as AS01 [19]. Adjuvant down-selection in mice and rhesus macaques showed that FMP013 combined with the novel Army Liposome Formulation (ALF) containing QS21 (ALFQ) adjuvant was ideal for inducing a potent immune response [20]. ALFQ is composed of anionic liposomes formed by phospholipids, a synthetic monophosphoryl lipid A analog, 3D-PHAD®; and the immune stimulant QS-21 [21–23]. The cholesterol concentration in ALFQ was optimized to reduce QS-21 reactogenicity allowing for higher doses of QS-21 to be used than AS01 [21]. In rhesus monkeys, ALFQ has been shown to elicit high levels of antigen-specific IFN- γ /IL-2 double-producing and poly-functional T cells [23–25]. Extensive repeat dose toxicity studies with FMP013/ALFQ were performed in mice, rhesus macaques and rabbits prior to advancing the FMP013/ALFQ vaccine to humans [18,20,26,27]. We present the first-in-human Phase-1 trial conducted to assess the safety and immunogenicity of the FMP013/ALFQ malaria vaccine candidate.

2. Material and methods

FMP013/ALFQ vaccine and vaccination: The antigen (FMP013) and the adjuvant (ALFQ) components of the vaccine have been described previously [20]. A cGMP lot of the FMP013 antigen was manufactured at the WRAIR Pilot Bioproduction Facility, protein bulk was stored at -80°C , and formulated, lyophilized at 60 μg

per vial in the final containers, stored at 4°C . Each mL of the drug product contained 0.1 mg of FMP013. Liposomes containing 55 mol % cholesterol (ALF55) were manufactured by Avanti Polar Lipids (Alabaster, AL). ALF55 was filter sterilized and QS-21 (Desert King, San Diego CA) was added, mixed to a homogenous solution, and aliquoted into 3 mL vials. A 1 mL ALFQ solution contains: 200 μg 3-deacyl monophosphoryl lipid A (3D-PHAD®), 7 mg 1,2-dimyristoyl-glycero-3-phosphocholine (DMPC), 0.78 mg 1,2-dimyristoyl-glycero-3-phosphoglycerol (DMPG), 5.4 mg cholesterol and 100 μg QS-21. Both FMP013 and ALFQ are stored at 4°C , mixed at bedside, and were stable for at least 4 h post-formulation.

Study design: This investigation was a Phase 1, open-label, single center study. The primary objective was to assess the safety and reactogenicity of FMP013/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. 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 NCT04268420).

Three vaccinations were administered in a dose-escalation trial design utilizing sentinel participants with doses ranging from a 20 μg FMP013/0.5 mL ALFQ (Low dose group) to 40 μg FMP013/1.0 mL ALFQ (High dose group) at study days 1, 29, and 57, with the last in-person visit on day 169 (Fig S1). 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.

Study Subjects: Subjects were malaria non-immune males and non-pregnant, non-lactating females aged 18–55 years (inclusive) from the Baltimore–Washington area (Supplementary Table S1). To be eligible for inclusion, participants had to be able to comply with study procedures and be in generally good health. They were assessed for serious acute or chronic illnesses by screening laboratory tests, complete medical history, and physical examination. Potential participants were excluded if they were immunosuppressed, had serological evidence of HBV, HCV, or HIV, a history of malaria vaccination, or recent malaria exposure. Full inclusion, exclusion and elimination criteria are listed in Appendix A of Supplementary materials.

Safety assessments: Safety and reactogenicity were assessed by collecting solicited local and systemic adverse events through 7 days post-vaccination and unsolicited adverse events and serious adverse events (SAEs) through the final study visit. The safety assessments were conducted with the use of memory aids and in-person follow-up visits 1, 2, 6 and 14 days after each vaccination. Safety labs consisted of a complete blood count (CBC) and serum chemistry including liver function, and were drawn prior to each vaccination. Solicited adverse events and labs were graded by study investigators according to the scale listed in Appendix B of supplementary material. Notably, a grade of 3 indicated a severity preventing everyday activity, redness measured $> 10\text{ cm}$ or temperature $> 39.0^{\circ}\text{C}$. The causality relationship of adverse events and vaccinations were graded as depicted in Appendix C. Phone visits were completed on study days 225 and 393.

3. Immunogenicity assessments:

Serologic assessments: ELISA and avidity assay against recombinant full-length CS protein (FL-CSP), repeat peptide (NANP)₆, or the C-terminal peptide Pf16 (Appendix D) were performed on sera samples collected on day: -7 (Prebleed), day 29 (4WP1), day 56 (4WP2), 71 (2WP3), and day 169 (16WP3), using standardized protocols by

the Malaria Serology Lab, WRAIR [15,18,28,29]. ELISA titer was defined as the serum dilution that resulted in OD = 1; and avidity index was the percentage of antibodies that remain bound following urea wash. An ELISA was also performed against synthetic peptides representing the N-terminal region CSP sequences – Peptide DNA: DNAGTNLYNELEMNYYGKQENWYSLKKNSRSLGEND; Peptide DGN: DGNNEDEKLRKPKHKKLKQP; Peptide KQP: KQPADGNPDPNANPNVDPN and Peptide KKK: KKKQPADGNPDPNANPNVDPNANPNVDPNANPNVDP. A subclass ELISA was performed using the FL-CSP coat antigen and subclass-specific secondary antibodies were used at 1:4000 dilution, essentially as described previously [18].

Functional assays: An inhibition of liver-stage development assay (ILSDA) was performed using NF54 *Pf* sporozoites incubated with sera from the 2WP3 time-point from each immunized individual along with the corresponding pre-bleed tested at 1:100 dilution. *Pf* 18S rRNA levels were determined using a quantitative real-time PCR and percent inhibition invasion was calculated for pre- and 2WP3 serum samples [30]. A multiplex assay Meso Scale Discovery (MSD Inc., Gaithersburg, MD) assay was used to measure the relative reactivity of serum samples to C-terminal peptides (CSP amino acid # 283–375; **Appendix D**), derived from 3D7 and seven non-3D7 CSP allele peptides [31,32]. Opsonophagocytosis activity (OPA) of the immune sera were determined as described previously [28]. The OPA index was defined as the log ratio of the OPA titer and the ELISA titer.

Frequency of cytokine producing cells: Fluorospot cytokine analysis was performed on PBMC samples collected on day –7 (Pre-bleed) and 71 (2WP3). The cells were thawed and stimulated with a CSP peptide mega pool (1 µg/mL) [33]. Frequency of antigen-specific interferon (IFN)-γ, interleukin 2 (IL-2) and TNF-α secreting T cells was measured by Fluorospot (Mabtech Inc., Cincinnati, OH) following the manufacturer's instructions [27]. Fluorospot plates were analyzed using the Autoimmun Diagnostica GmbH Fluorospot reader (Strassberg, Germany) and data expressed as spot forming cells (SFCs)/10⁶ PBMCs. Th1 and Th2

cytokines (IFNγ, IL1, IL2, IL4, IL5, IL6, IL8, IL10, IL12p70, TNF-α) were profiled using MSD multiplex testing platform. PBMCs from day –7 (pre) and day 71 (2WP3) were stimulated with CSP peptide mega pool (1 µg/mL). Medium-only was the negative control and anti-CD3 mAb was used as the positive control [34]. A V-PLEX Human Proinflammatory Panel was used to quantitate the cytokines using a MESO QuickPlex SQ120 and expressed as pg/mL per 10⁶ PBMCs.

Statistical analyses: All safety analyses were performed on the intent-to-treat (ITT) group and were descriptive in nature. Immunologic analyses and comparisons to the benchmark were performed on the according-to-protocol (ATP) population. All significant differences were assessed by ANOVA followed by Tukey's Multiple Comparison Correction or Sidak's test. Two-way comparisons were made using a 2-tailed T-test and P < 0.05 was used as a cutoff for statistical significance. All statistical analyses were conducted using Prism Graphpad Version 9 or Minitab V18 (Minitab LLC, State College, PA).

4. Results

Enrollment: A total of 10 subjects were enrolled, 5 in the Low dose group and 5 in the High dose group. 80% of the Low dose and 40% of the High dose volunteers were female (**Supplementary Table S1**). The mean participant age was 28.4 years and 26.8 years for the Low and High dose groups, respectively. Overall, 7 of the 10 subjects completed all 3 vaccinations (**Fig. 1**). One subject from the High dose group was withdrawn after the first vaccination, and one subject each from the High dose and Low dose groups was withdrawn after the second vaccination.

Safety and tolerability: High and Low doses of FMP013/ALFQ were well tolerated with similar mild reactions at the injection site experienced in a majority of volunteers after each vaccination. The solicited adverse events are listed in **Table 1** and plotted in **Fig. 2**. In the Low dose group, systemic reactions were mild or not present

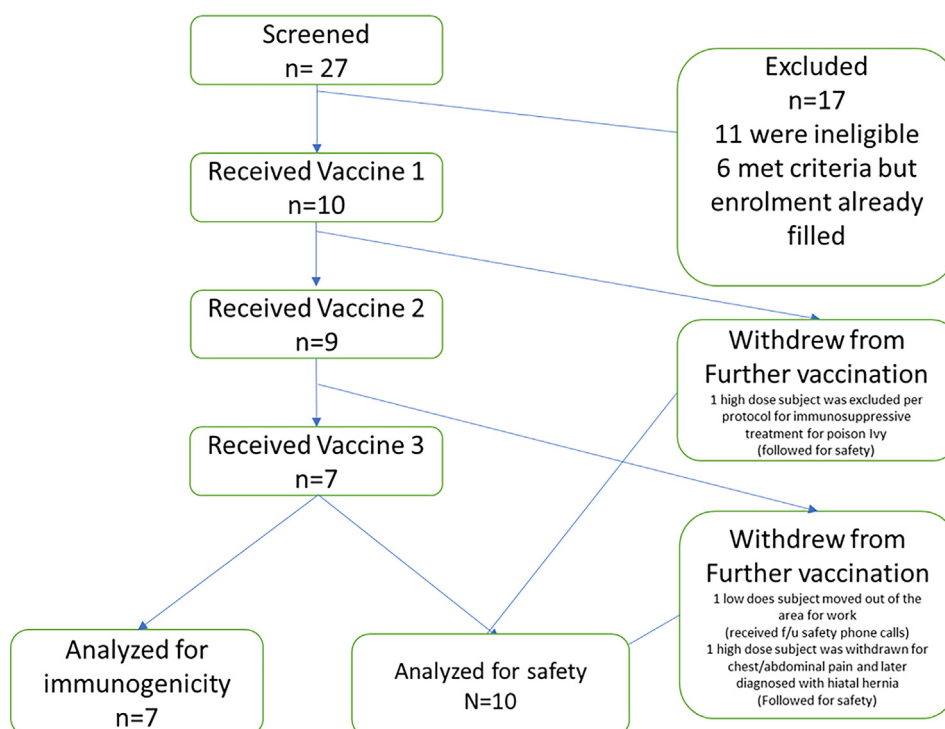


Fig. 1. Consolidated Standard of Reporting Trials flow diagram: Number of subjects that underwent screening enrollment, vaccinations and analysis as well as those that withdrew from the study are shown.

Table 1
Solicited AE's by dose and vaccination. Number of subjects experiencing each severity of AE are shown. Only the highest severity grade for each vaccination was selected.

	Low Dose 1	High Dose 1	Low Dose 2	High Dose 2	Low Dose 3	High Dose 3
Injection site redness	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 1 Mild n = 1 Moderate n = 1 Severe n = 0
Injection site Pain	None n = 0 Mild n = 4 Moderate n = 1 Severe n = 0	None n = 0 Mild n = 5 Moderate n = 0 Severe n = 0	None n = 0 Mild n = 5 Moderate n = 0 Severe n = 0	None n = 0 Mild n = 2 Moderate n = 2 Severe n = 0	None n = 1 Mild n = 3 Moderate n = 0 Severe n = 0	None n = 0 Mild n = 3 Moderate n = 0 Severe n = 0
Chills	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 5 Mild n = 1 Moderate n = 0 Severe n = 0	None n = 1 Mild n = 3 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 0 Mild n = 1 Moderate n = 2 Severe n = 0
Fatigue	None n = 2 Mild n = 3 Moderate n = 0 Severe n = 0	None n = 2 Mild n = 3 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 1 Moderate n = 0 Severe n = 0	None n = 0 Mild n = 3 Moderate n = 1 Severe n = 0	None n = 3 Mild n = 1 Moderate n = 0 Severe n = 0	None n = 1 Mild n = 1 Moderate n = 1 Severe n = 0
Headache	None n = 3 Mild n = 2 Moderate n = 0 Severe n = 0	None n = 2 Mild n = 3 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 1 Moderate n = 1 Severe n = 0	None n = 1 Mild n = 2 Moderate n = 1 Severe n = 0	None n = 4 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 1 Mild n = 0 Moderate n = 2 Severe n = 0
Myalgias	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 3 Mild n = 1 Moderate n = 1 Severe n = 0	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 1 Mild n = 2 Moderate n = 1 Severe n = 0	None n = 3 Mild n = 1 Moderate n = 0 Severe n = 0	None n = 0 Mild n = 1 Moderate n = 2 Severe n = 0
Arthralgias	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 3 Mild n = 2 Moderate n = 0 Severe n = 0	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 0 Mild n = 4 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 1 Mild n = 1 Moderate n = 1 Severe n = 0
Nausea	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 1 Moderate n = 0 Severe n = 0	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 3 Mild n = 1 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 2 Mild n = 1 Moderate n = 0 Severe n = 0
Pyrexia	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 3 Mild n = 1 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 2 Mild n = 1 Moderate n = 0 Severe n = 0
Diarrhoea	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 1 Moderate n = 0 Severe n = 0	None n = 5 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 3 Mild n = 1 Moderate n = 0 Severe n = 0	None n = 4 Mild n = 0 Moderate n = 0 Severe n = 0	None n = 3 Mild n = 0 Moderate n = 0 Severe n = 0

after first vaccination and remained scarce with subsequent vaccinations. In the High dose group systemic reactions were typically mild after the first dose but trended towards moderate with the subsequent two vaccine doses, predominantly manifesting as headache, fatigue, and/or chills. Most solicited AEs manifested at approximately 36 h and typically resolved by 72 h post-vaccination. There were no severe solicited AEs and measured fevers were only observed in a minority of the High dose group. No deaths or serious adverse events were reported. Lab abnormalities were generally mild in nature and had no association with dose or timing of vaccination. Unsolicited or potentially related adverse events, listed in Table 2, were minimal and mild with the exception of the positional pleuritic discomfort stemming from a hiatal hernia of a High dose subject who was withdrawn after the second vaccination. One subject in the low dose group had prolonged mild neck muscle stiffness and sporadic episodes of once per day loose stools after the first vaccination that resolved before the second vaccination and did not return. Overall, both the High and Low dose of FMP013/ALFQ were well tolerated with a possible trend of increasing reactogenicity with the higher dose.

Serology: After the first vaccination, all subjects seroconverted (ELISA OD = 1 titer > 100) against the full-length recombinant CSP (FL-CSP), NANPx6 (repeat peptide) and Pf16 (C-terminal region peptide) coat antigens (Fig. 3A, B). The second dose of FMP013/ALFQ boosted geometric mean FL-CSP titers 13- to 39-fold in the High and Low dose groups respectively, while the third dose

boosted titer ~ 2-fold in both groups, the increase was not statistically significant. The boosting of titers post 3rd vaccination was lost within 16 weeks as the titers receded against all 3 plate antigens in both dose groups. The ratio of the Pf16 to NANPx6 ELISA titer increased 3- to 4- fold after the second and third vaccine doses, sharply skewing the antibody ratio towards the C-terminus (Fig. 3C).

To compare the immunogenicity between the groups, the day 71 and day 169 titers and avidity for individuals who received all three vaccinations according to protocol were analyzed (Fig. 4). The day 71 NANPx6 repeat, C-terminal region and FL-CSP geometric mean titers were 1.5-fold, 3.2-fold and 2.2-fold greater, respectively, than the Low dose, but this difference did not reach statistical significance. On day 169, the geometric mean FL-CSP and C-terminal titer for the High dose group were significantly greater than the Low dose group, suggesting the High dose vaccine was more immunogenic (Fig. 4A). Avid antibody binding against all three plate antigens was observed on day 71, with no significant difference between groups (Fig. 4B). Antibody avidity levels were sustained through day 169.

Peptides listed in Appendix D were used to map N-terminal region responses by ELISA. Day 71 sera showed no reactivity to N-terminal peptides DNA, DGN, however peptides KQP and KKK that encompassed the Region I and the junctional sequence showed positive reactivity (Supplementary Fig S2A). The N-terminal region titers were however much lower than NANPx6

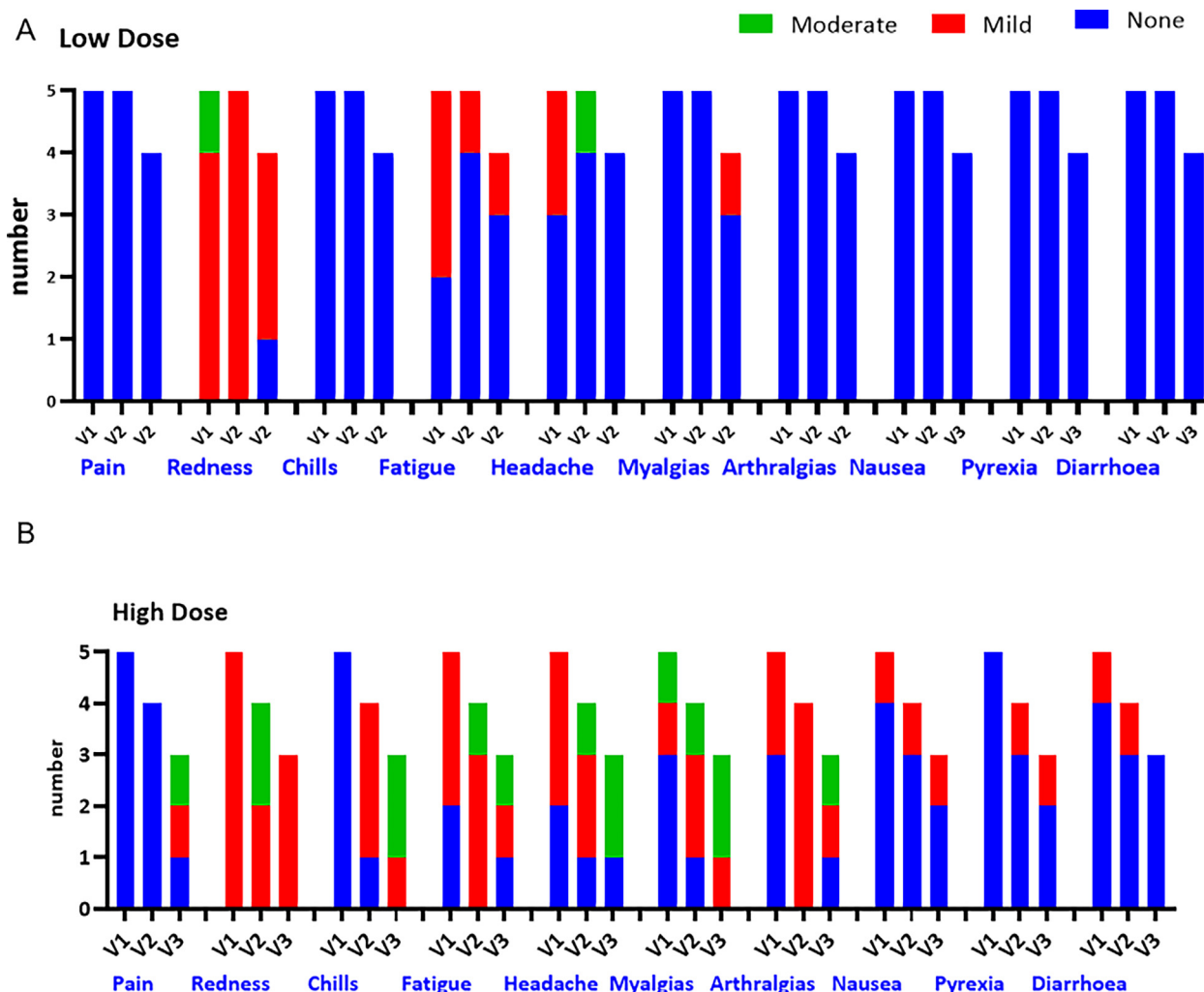


Fig. 2. Solicited AE's by dose and vaccination. Number of subjects experiencing each grade of AE are shown. Only the highest grade for each vaccination was selected. All observed events were graded mild or moderate and there were no severe adverse events.

Table 2

Listing of Unsolicited Potentially Related AEs by Dose and Vaccination. The list excludes unlikely, and not related unsolicited AEs, Listed peak severity by each preferred term, each vaccination and each subject.

Treatment Group	Dose	Preferred Term	Relatedness	Severity	Day of AE Start After Vaccination	Duration	Outcome
Low Dose	1	Diarrhoea	Possible	Mild	3	1	Recovered/Resolved
Low Dose	1	Diarrhoea	Possible	Mild	12	17	Recovered/Resolved
Low Dose	1	Musculoskeletal stiffness	Possible	Mild	2	21	Recovered/Resolved
High Dose	1	Diarrhoea	Possible	Mild	2	2	Recovered/Resolved
High Dose	1	Pleuritic pain	Possible	Severe	9	11	Recovered/Resolved
High Dose	2	Night sweats	Possible	Mild	18	4	Recovered/Resolved
High Dose	2	Night sweats	Probable	Mild	1	1	Recovered/Resolved
High Dose	2	Pyrexia	Possible	Moderate	2	1	Recovered/Resolved
High Dose	2	Respiratory tract congestion	Possible	Mild	1	1	Recovered/Resolved
High Dose	3	Abnormal dreams	Probable	Mild	1	2	Recovered/Resolved
High Dose	3	Dizziness	Probable	Mild	1	2	Recovered/Resolved
High Dose	3	Subjective Fever	Probable	Mild	1	2	Recovered/Resolved
High Dose	3	Subjective Fever	Probable	Moderate	1	3	Recovered/Resolved

and Pf16 peptide titers (Fig. 4), suggesting N-terminal region antibody response to be relatively weak. Immunoglobulin subclass ELISA performed using day 71 sera, showed predominantly IgM and high levels of IgG1 and IgG3 (Supplementary Fig S2B).

Functional responses: Serum samples at day 71 inhibited sporozoite invasion into human hepatocytes as measured by an ILSA at 1:100 serum dilution (Fig. 5A). Previous work on RTS,S

has revealed an inverse relationship between opsonophagocytosis assay (OPA) index and protection [28]. The Day 71 sera tested positive on this OPA (Fig. 5B). Previous work also demonstrated a positive correlation between wider C-terminal antibody breadth and protection using RTS-S-immune sera [32]. Day 71 sera tested for C-terminal cross-reactivity showed lower reactivity to heterologous peptides (H234, H18, H3, H1, H12, H14, H50) as compared

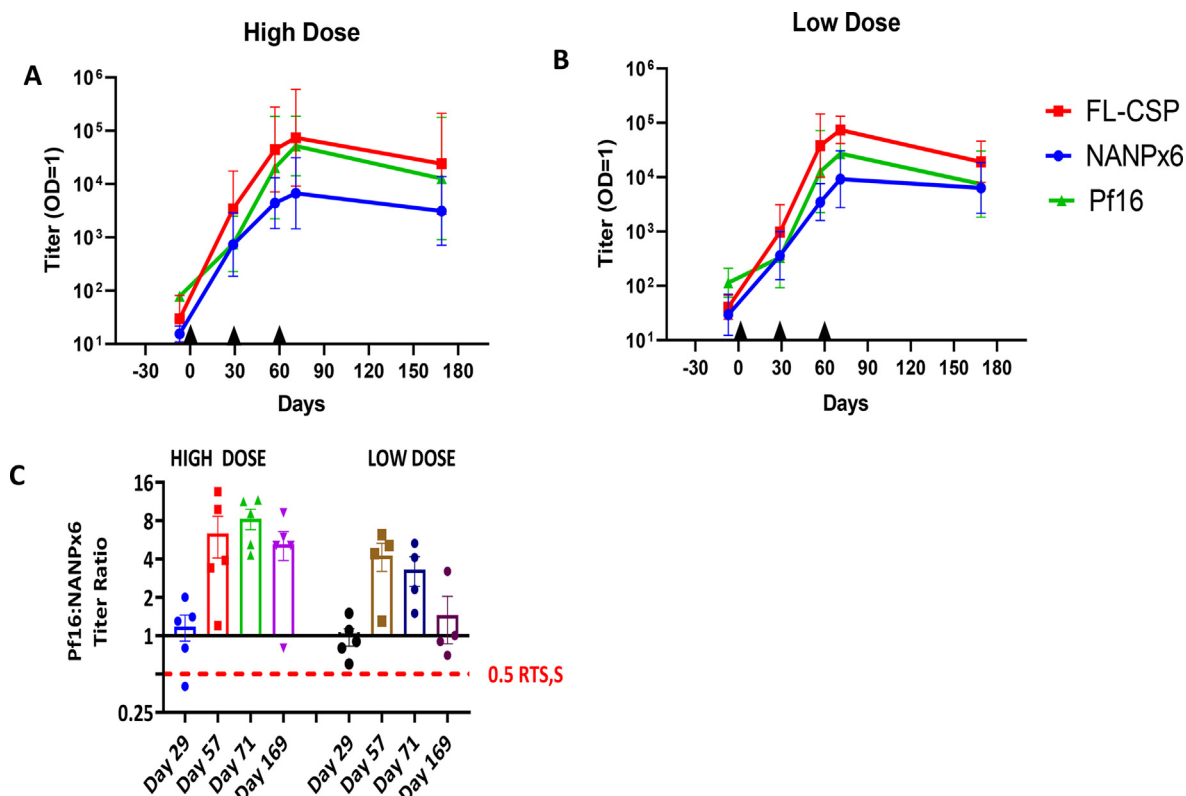


Fig. 3. Serological testing. A, B) Geometric mean ($\pm 95\%$ Confidence interval) for all subjects (Intent to treat) measured by FL-CSP, NANPx6 and Pf16 ELISA following three vaccinations with the High or Low FMP013/ALFQ (arrows). C) The ratio of Pf16/NANPx6 titer for all subjects. The red dotted line is the average Pf16/NANPx6 titer ratio for individuals who received 3 standard vaccinations of RTS,S/AS01 as part of the MAL071 trial [15].

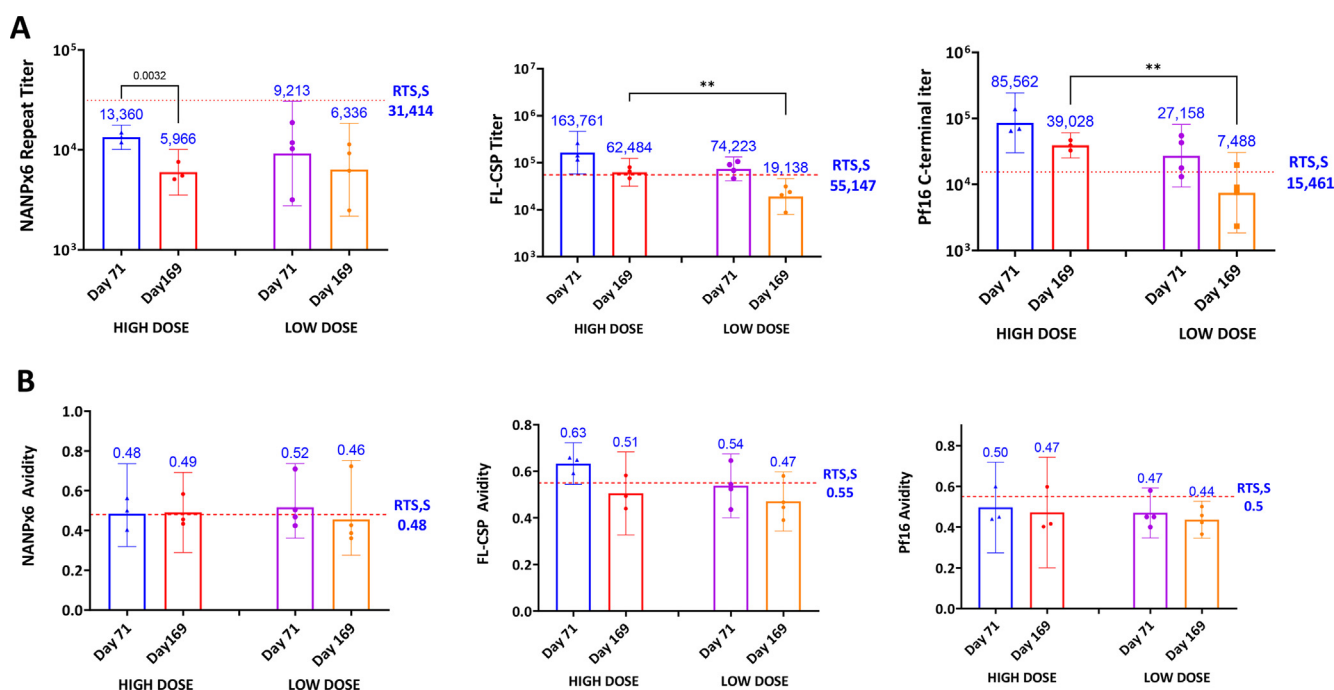


Fig. 4. A) Geometric mean ($\pm 95\%$ CI) titer and **B)** mean avidity on day 71 and 169 for individuals who received all 3 vaccinations (per protocol) against the NANPx6, FL-CSP and the Pf16 C-terminal peptide. RTS,S MAL071 standard dose benchmarks are shown as red dotted lines. The P values for unpaired T tests are shown.

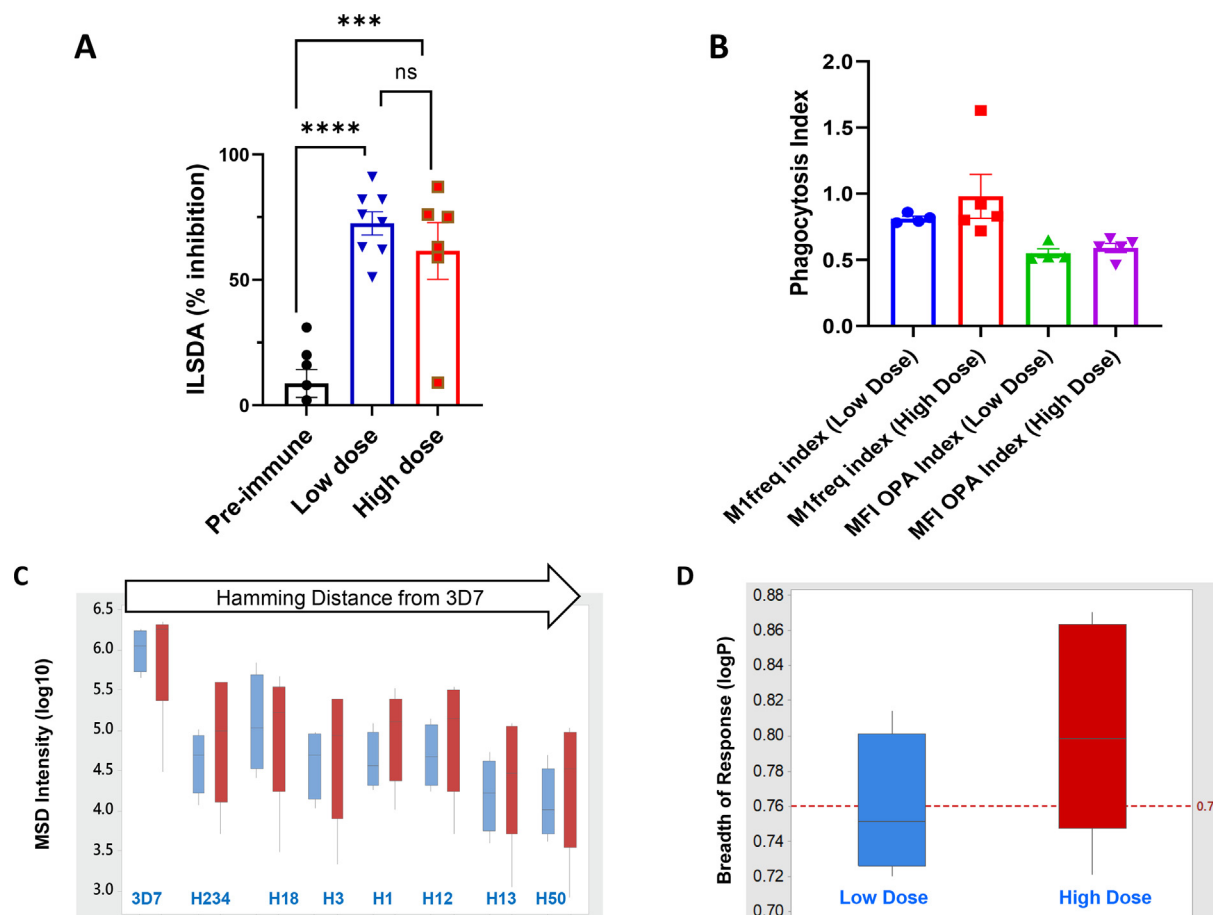


Fig. 5. Functional Analysis. **A)** Percent inhibition of invasion measured by ILSDA at 1:100 serum dilution (data from two independent experiments). **B)** Mfreq and MFI opsonization index of low and high dose vaccine sera; **C)** Serum reactivity of post-immune sera with 3D7 and seven variant CSP C-terminal peptides. Box plots summarize reactivity of 9 vaccinees (Blue box = Low dose, red box = High dose). Data expressed as net luminescence signal (pre-immune signal was subtracted; mean luminescence signal of CSP-negative sera 480 ± 90). Data ordered in increasing sieve hamming distance, left to right. **D)** Breadth of the serum response (blue = Low dose; red = High dose) to C-terminal CSP peptides expressed as median response across all tested variant peptides relative to 3D7. Dotted line was the reference average breadth observed with RTS,S-immune samples [32].

to the homologous 3D7 strain peptide (Fig. 5C). A trend towards improved overall breadth of C-terminal region antibody responses was also observed for the High dose group (Fig. 5D).

T-cell responses: The MSD analysis (Fig. 6A, 6B) revealed that in the high dose group, Th1 cytokines (IL-2, IFN- γ and TNF- α) but not Th2 cytokines (IL-4, IL-13, IL-10), were induced after peptide stimulation in the PBMC cultures (Supplementary Table S2). In the low dose group, only the IFN- γ levels above pre-immune were observed. We proceeded to monitor changes in the frequency of PBMCs producing these Th1 cytokine responses by Fluorospot. In both the Low and High dose group the day 71 frequency of CSP-specific IFN- γ , IL-2 and TNF- α positive PBMCs, showed significantly higher SFC/10⁶ cells compared to the corresponding media control wells (Fig. 6C, D).

5. Discussion/Conclusion

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 FMP013 antigen and the ALFQ adjuvant demonstrated an acceptable safety and tolerability profile. FMP013/ALFQ elicited antibodies that bound to the repeat and C-terminal peptides and low-level anti-N-terminal Region I and anti-junctional peptide antibodies were elicited. Sera inhibited

the invasion *P. falciparum* sporozoites and possessed positive opsonophagocytosis activity. Consistent with responses observed during pre-clinical animal studies, FMP013/ALFQ elicited a Th1-biased cytokine response. Together, these data supported the follow-on evaluation of this vaccine candidate in a CHMI study.

Due to compressed timelines during the Covid-19 pandemic, the safety monitoring committee (SMC) made their dosing recommendation based on the day 71 serology data from the intention to treat group. There was a trend of increased but acceptable reactivity in the higher dose group. Limited immunogenicity data from a small number of subjects in the intention-to-treat group suggested that the difference between high and low dose immunogenicity was not statistically significant, hence the SMC recommendation was to proceed to the subsequent CHMI trial with the Low dose. Here a per-protocol analysis, in a very small cohort size, shows a consistent trend that the High dose titers on day 71 and 169 were consistently higher than the Low dose (Fig. 4A). Future trials to optimize the antigen and adjuvant dose for the FMP013/ALFQ formulation need to be conducted.

While RTS,S sera were not available for direct comparison in assays, the FMP013/ALFQ immunogenicity data were compared to previously reported benchmarks from RTS,S clinical trials. Specifically, the geometric mean titer of volunteers who received 3x50 μ g RTS,S in 0.5 mL AS01_B as reported by Regules *et al.* [15]

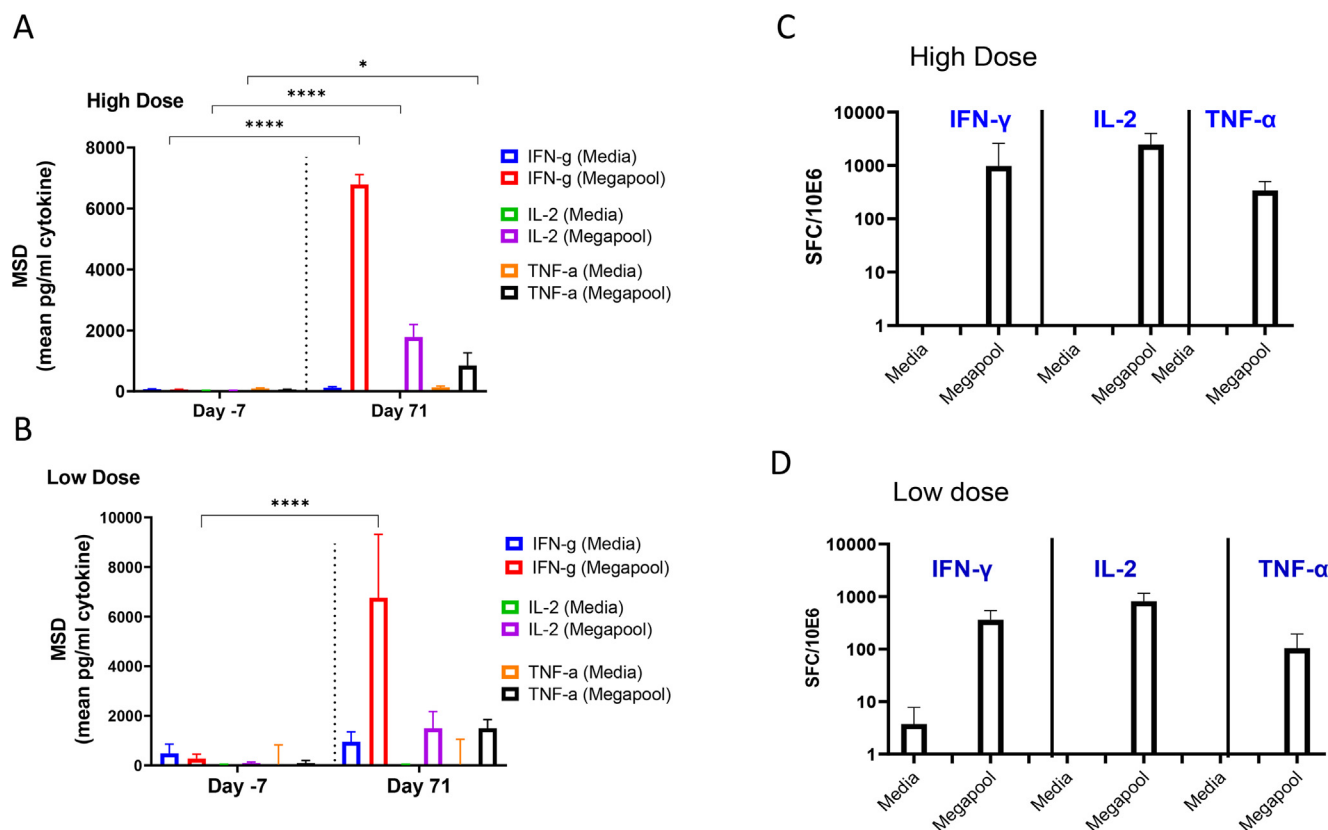


Fig. 6. T-cell responses: A, B Th1 cytokines measured using Mesoscale multiplex assay following stimulation with CSP peptide pool. Mean IFN- γ , IL-2 and TNF- α levels (pg/ml) subtracted from the pre-immune controls are shown for day -7 (pre-immune) and day 71. P values were corrected by Sidak's test for multiple comparisons. **C, D** Frequency of CSP-specific IFN- γ , IL-2 and TNF- α producing lymphocytes subtracted from the pre-immune controls and assessed by Fluorospot following stimulation with CSP peptide pool. All responses were significantly higher than medium controls.

were plotted as red dotted lines in Fig. 4. The NANPx6 repeat region titer for RTS,S/AS01 was 2 and 3-fold greater than the corresponding High and Low dose FMP013/ALFQ titers at day 71, respectively (Fig. 4A). In sharp contrast, the respective C-terminal region titers of FMP013/ALFQ High and Low dose groups were 3 and 1.9-fold greater than RTS,S/AS01, respectively. Likewise, FMP013/ALFQ FL-CSP titers exceeded the RTS,S/AS01 benchmark by 3- and 1.3-fold in the High and Low dose groups respectively. The day 71 avidity measurements of FMP013/ALFQ against FL, Repeat and C-term antigens were comparable to RTS,S/AS01 (Fig. 4B). IgG1 and IgG3 were the predominant subclasses elicited by FMP013/ALFQ (Supplementary Fig S2B), both of which have been associated with RTS,S mediated protection [35]. OPA activity described previously for RTS,S immune sera was comparable to RTS,S [36]. The IFN- γ , IL-2 and TNF- α Fluorospot frequency on day 71 for FMP013/ALFQ recipients compared favorably to the equivalent ELISpot frequencies reported for volunteers receiving RTS,S [37]. While only head-to-head comparisons of vaccines can be definitive, our data show that the titers elicited by FMP013/ALFQ were higher than those elicited by a similar full-length CSP construct adjuvanted with GLA/LSQ [38].

As compared to RTS,S/AS01_B, the FMP013/ALFQ induced a highly C-terminal region biased response (Fig. 3C). We have previously shown that particulate presentation of CSP selectively boosts the immunogenicity of its long and flexible repeat region [39]. The particulate nature of RTS,S may also be focusing its immunogenicity towards the repeat region, while a soluble protein like FMP013 may be eliciting higher titers to the more structured C-terminal region. The moderate efficacy of RTS,S has been partly attributed to strain mismatch between the 3D7 strain C-terminal and the par-

asites circulating in the field [17,32]. Hence, there is a concern that a C-terminal biased vaccine may not be effective in the field. We found that the C-terminal antibody breadth of the High dose group of FMP013 recipients exceeded the historical benchmark of RTS,S reported previously (Fig. 5D)[32]. The augmented C-terminal region antibody response and higher cross-reactivity to heterologous C-terminal region peptides suggests that FMP013/ALFQ could be more effective than RTS,S/AS01 against the diversity of *Plasmodium falciparum* parasite strains in the field [32].

Early CSP vaccines based on soluble protein and peptides elicited little to no protection in humans [40], resulting in the prevailing view that particle based antigens may be necessary to elicit high-level protection against malaria. However, particulate RTS,S antigen, combined with alum or monophosphoryl lipid A (MPL) containing adjuvants also showed poor efficacy in early trials [19]. By contrast, RTS,S, formulated with immune-stimulants MPL and QS21, first the oil-in-water emulsion AS02 and then the liposomal formulation AS01, reproducibly protected ~ 50% of vaccinees in CHMI trials [41–44]. Indeed, the soluble protein herpes zoster vaccine (ShingrixTM) adjuvanted with AS01 is proof that soluble protein vaccines can be highly efficacious [45]. Soluble antigens offer a more cost-effective scale-up and improved stability compared to the structurally complex particulate antigens. Improved efficiencies in cost will be impactful for real-world deployment of malaria vaccines [46]. Notably, soluble CSP-based FMP013, formulated in adjuvants Alum, GLA/SE or conjugated to the Q β phage, did not meet the progression benchmark in rhesus macaque studies [18,39,47]. The benchmark criteria was only met when FMP013 was combined with ALFQ [27,26]. These current human data there-

fore also validate the use of the rhesus macaque model as a stage gate for progression of next-generation CSP vaccines to humans.

Declaration of Competing Interest

The authors JNH, LS, KM, VN, PR, EA, DEL, JEM, NCW, MH, HG, CL, LZ, XQ, DRB, ED, JB, XZ, EB, JR declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors SD, ZB, and GM declare the following financial interests which may be considered as potential competing interests: SD holds a patent on the FMP013 antigen; SD, ZB, GM have filed a patent for the FMP013/ALFQ formulation. The material has been reviewed by the Walter Reed Army Institute of Research and the US Agency for International Development. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army, the Department of Defense, or the US Agency for International Development.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

Designed the study: LS, JR, SD, JNH, KM, VN, PR, EA, DEL, JEM, NCW. *Conducted the study:* JEM, MH, HG, CL, LZ, XQ, DRB, ED, JB, XZ, EB, JR, SD, JNH, KM, VN, PR. *Wrote the paper:* JR, SD, JNH, KM, EB. *Developed reagents:* GM, ZB, SD.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2022.08.048>.

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