

# Serologic Markers of Previous Malaria Exposure and Functional Antibodies Inhibiting Parasite Growth Are Associated With Parasite Kinetics Following a *Plasmodium falciparum* Controlled Human Infection

Jane Achan,<sup>1,a</sup> Isae j. Reuling,<sup>2,a</sup> Xi Zen Yap,<sup>2</sup> Edgard Dabira,<sup>1</sup> Abdullahi Ahmad,<sup>1</sup> Momodou Cox,<sup>1</sup> Davis Nwakanma,<sup>1</sup> Kevin Tetteh,<sup>3</sup> Lindsey Wu,<sup>3</sup> Guido J. H. Bastiaens,<sup>2</sup> Yonas Abebe,<sup>4</sup> Anita Manoj,<sup>4</sup> Harparkash Kaur,<sup>3</sup> Kazutoyo Miura,<sup>5</sup> Carole Long,<sup>5</sup> Peter F. Billingsley,<sup>4</sup> B. Kim Lee Sim,<sup>4</sup> Stephen L. Hoffman,<sup>4</sup> Chris Drakeley,<sup>3</sup> Teun Bousema,<sup>2,b</sup> and Umberto D'Alessandro<sup>1,b</sup>

<sup>1</sup>Disease Control and Elimination Theme, Medical Research Council Unit The Gambia at London School of Hygiene and Tropical Medicine, Banjul, The Gambia; <sup>2</sup>Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands; <sup>3</sup>Department of Immunology and Infection, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, United Kingdom; and <sup>4</sup>Sanaria Inc, and <sup>5</sup>Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland

**Background.** We assessed the impact of exposure to *Plasmodium falciparum* on parasite kinetics, clinical symptoms, and functional immunity after controlled human malaria infection (CHMI) in 2 cohorts with different levels of previous malarial exposure.

**Methods.** Nine adult males with high (sero-high) and 10 with low (sero-low) previous exposure received 3200 *P. falciparum* sporozoites (PfSPZ) of PfSPZ Challenge by direct venous inoculation and were followed for 35 days for parasitemia by thick blood smear (TBS) and quantitative polymerase chain reaction. Endpoints were time to parasitemia, adverse events, and immune responses.

**Results.** Ten of 10 (100%) volunteers in the sero-low and 7 of 9 (77.8%) in the sero-high group developed parasitemia detected by TBS in the first 28 days ( $P = .125$ ). The median time to parasitemia was significantly shorter in the sero-low group than the sero-high group (9 days [interquartile range {IQR} 7.5–11.0] vs 11.0 days [IQR 7.5–18.0], respectively; log-rank test,  $P = .005$ ). Antibody recognition of sporozoites was significantly higher in the sero-high (median, 17.93 [IQR 12.95–24] arbitrary units [AU]) than the sero-low volunteers (median, 10.54 [IQR, 8.36–12.12] AU) ( $P = .006$ ). Growth inhibitory activity was significantly higher in the sero-high (median, 21.8% [IQR, 8.15%–29.65%]) than in the sero-low group (median, 8.3% [IQR, 5.6%–10.23%]) ( $P = .025$ ).

**Conclusions.** CHMI was safe and well tolerated in this population. Individuals with serological evidence of higher malaria exposure were able to better control infection and had higher parasite growth inhibitory activity.

**Clinical Trials Registration.** NCT03496454.

**Keywords.** malaria exposure; parasite kinetics; clinical outcomes; functional antibodies; controlled human malaria infection.

Naturally acquired immunity against malaria parasites, which limits high-density parasitemia and severe disease, develops after repeated exposure, and more rapidly in high- than in low-transmission areas [1, 2]. This immunity is thought to be primarily mediated by anti-blood stage antibodies, which reduce parasite multiplication and cytoadherence of infected erythrocytes to endothelial cells [3]. In contrast, there is limited evidence

for immunological responses preventing blood-stage infection by neutralizing sporozoites and liver-stage parasites [4, 5].

Over the past 2 decades, malaria control measures have led to substantial reductions in malaria burden [6], with several endemic countries transitioning from high to low malaria transmission [7, 8]. Decreased malaria exposure leads to increased susceptibility to infection and severe disease [9, 10] and is associated with decreased levels of antibodies to blood-stage antigens [11–13].

Controlled human malaria infection (CHMI) of healthy volunteers by exposure to the bites of infected, laboratory-reared *Anopheles* mosquitoes or inoculation of infected erythrocytes has been used for nearly 100 years to investigate malaria pathophysiology and immunology and efficacy of vaccines and drugs [14, 15]. During the last decade, CHMI studies have been expanded in the United States and Europe and increasingly performed in Africa using injectable, aseptic, purified, cryopreserved, vialled *Plasmodium falciparum* sporozoites (PfSPZ, Sanaria PfSPZ Challenge) [5, 16–20], including assessment of innate resistance

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<sup>a</sup>J. A. and I. R. contributed equally to this work.

<sup>b</sup>T. B. and U. D. contributed equally to this work.

Correspondence: J. Achan, MRC Unit The Gambia at London School of Hygiene and Tropical Medicine, PO Box 273, Banjul, The Gambia (jachan@mrc.gm).

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[5], naturally acquired immunity, and preerythrocytic and asexual erythrocytic-stage vaccines [20, 21]. In this study we assessed how exposure to *P. falciparum*, as measured by serology to 6 predefined antigens, affected parasite kinetics, clinical symptoms, and functional immunity after CHMI by direct venous inoculation (DVI) of PfSPZ Challenge [16, 17] in Gambian men with markedly different levels of previous malarial exposure.

## METHODS

### Study Design and Participants

This was an open-label, nonrandomized clinical trial, conducted at the Medical Research Council Unit The Gambia (MRCG). Healthy male participants aged 18–35 years were recruited between 13 and 23 March 2018. Volunteers were preferentially recruited from tertiary learning institutions and provided written informed consent before screening. Eligible volunteers had normal hematological and biochemical tests and no abnormalities by electrocardiography. Participants had to be *P. falciparum* negative by molecular methods on 2 occasions, at recruitment and just before DVI. Previous individual *P. falciparum* exposure was assessed using serologic responses to a panel of *P. falciparum* antigens using a Luminex platform [22]. These included responses associated with cumulative exposure, namely apical membrane antigen 1 (AMA-1), merozoite surface protein 1.19 (MSP-1.19), and glutamate-rich protein (GLURP.R2) [23], and responses associated with malaria infection in the past 6 months, namely reticulocyte-binding protein homologue (Rh2.2030), gametocyte exported protein (GEXP18), and early transcribed membrane protein (Etramp5.Ag1) [24]. A complete description of the eligibility criteria is provided in [Supplementary Appendix 1](#). The study received approval from the Scientific Coordinating Committee of MRCG, The Gambia Government/MRCG Joint Ethics Committee, and the London School of Hygiene and Tropical Medicine Research and Ethics Committee and was conducted according to the International Conference on Harmonisation Good Clinical Practice guidelines and registered with ClinicalTrials.gov (identifier NCT03496454).

### Study Objectives

The primary objectives were to assess the feasibility of the CHMI model in The Gambia and determine the parasite kinetics in naturally exposed Gambian adults after PfSPZ Challenge administration. Secondary objectives were to analyze humoral and cellular immune responses and their association with time to patency and parasite density at time of first detection, and to assess the frequency, incidence, nature, and magnitude of adverse events.

### PfSPZ Challenge

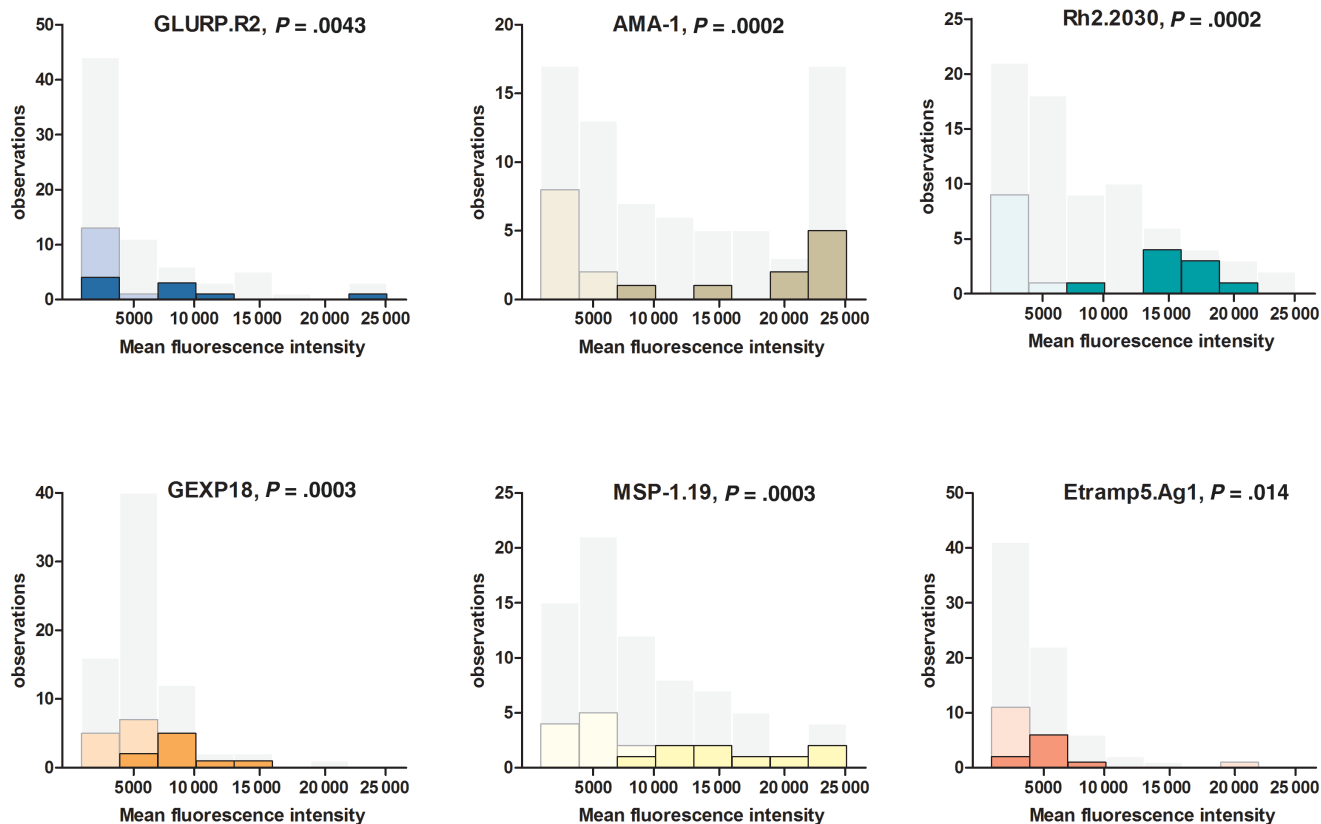
Sanaria PfSPZ Challenge is composed of aseptic, purified, vialled, cryopreserved, fully infectious NF54 PfSPZ isolated

from *Anopheles stephensi* mosquitoes [16, 18, 25]. PfSPZ Challenge was supplied by Sanaria Inc as 20- $\mu$ L cyrovials containing 15 000 PfSPZ and stored in liquid nitrogen vapor phase at  $-150^{\circ}\text{C}$  to  $-196^{\circ}\text{C}$  [25]. For this study, only 1 lot of PfSPZ manufactured on 30 April 2015 was used. The potency (capacity to invade and fully develop in cultured human hepatocytes [HC-04]) and viability (sporozoite membrane integrity) of this lot were tested as detailed in [Supplementary Appendix 2](#).

### Study Procedures

All screened volunteers were ranked by the cumulative quartile score of the mean fluorescence intensities of the 6 predefined antigens [24]. Volunteers with the highest and lowest scores were assigned to the sero-high and sero-low groups, respectively. This classification resulted in significantly higher responses to all individual antigens reflected by mean fluorescence intensities of cumulative and recent exposure markers that were 4- to 13-fold and 3- to 5-fold higher, respectively, in the sero-high group ([Supplementary Appendix 3](#)). While populations were defined based on a cumulative quartile score for all antigens combined, recognition was also statistically significantly higher for the high exposure population for each of the 6 individual antigens ( $P < .014$ ) ([Figure 1](#)). All volunteers received PfSPZ Challenge ( $3.2 \times 10^3$  PfSPZ in 0.5 mL) by DVI through a 25-gauge needle performed on a single day (29 March 2018) following Sanaria's standard operating procedures. After injection, participants were observed for 1 hour and subsequently closely monitored on an outpatient basis, with regular visits to the study clinic. Participants were instructed to register their daily symptoms in a study diary, measure temperature twice daily, and contact the clinical investigators when any symptoms occurred. From day 5 postinjection onward, participants were seen twice daily until day 15, and daily until day 28 or day of treatment. At each follow-up visit, temperature was taken, adverse events (AEs) were recorded, and blood samples were collected; physical examination was done on indication. Participants had a mobile phone by which they could be contacted. As an additional safety precaution, participants stayed in a hostel close to the study clinic from the day of infection until 3 days after treatment. The following signs and symptoms were solicited at all visits: fever, headache, malaise, fatigue, dizziness, myalgia, arthralgia, nausea, vomiting, chills, diarrhea, abdominal pain, chest pain, palpitations, and shortness of breath [26]. AEs were reported as mild (grade 1, easily tolerated), moderate (grade 2, interfered with normal activity), or severe (grade 3, prevented normal activity); for fever, as grade 1 ( $>37.5^{\circ}\text{C}$ – $38.0^{\circ}\text{C}$ ), grade 2 ( $38.1^{\circ}\text{C}$ – $39.0^{\circ}\text{C}$ ), or grade 3 ( $\geq 39.1^{\circ}\text{C}$ ). Laboratory values were graded using the National Institute of Allergy and Infectious Diseases Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 2.1, March 2017.

If a thick blood smear (TBS) was positive with any parasitemia, with or without signs and symptoms of malaria,



**Figure 1.** Antibody histogram plots for screened volunteers in The Gambia controlled human malaria infection study. Light colors are the sero-low group, dark colors are the sero-high group, and gray colors are the other screened volunteers with intermediate immunological profile. Abbreviations: AMA-1, apical membrane antigen 1; Etramp5. Ag1, early transcribed membrane protein; GEXP18, gametocyte exported protein; GLURP.R2, glutamate-rich protein; MSP-1.19, merozoite surface protein 1.19; Rh2.2030, reticulocyte-binding protein homologue.

treatment with artemether-lumefantrine was started immediately. Participants who did not develop parasitemia by day 28 received artemether-lumefantrine on that day. Treatment was directly observed, and all participants were seen at day 35 for an end of study visit.

#### Blood Sampling and Laboratory Assessments

Screening for parasitemia by microscopic examination of TBS and quantitative polymerase chain reaction (qPCR) was done twice daily from days 5 to 15 and daily from days 16 to 28. A complete blood count was done the day prior to PfSPZ Challenge injection, every 3 days between days 5 and 28, just before treatment, and thereafter daily for the following 3 days and at day 35. Blood biochemistry was performed 1 day before PfSPZ Challenge injection, 2 days after treatment, and at day 35. To check if volunteers had self-medicated with artemether-lumefantrine, lumefantrine levels were measured at baseline by high-performance liquid chromatography with photodiode array detection [27]. Peripheral blood mononuclear cells were collected for immunological studies 1 day before PfSPZ Challenge injection and at day 35. Malaria infection was defined as asexual parasites in peripheral blood by TBS during the

study and by qPCR retrospectively. The prepatent period was defined as the time between PfSPZ Challenge injection and first positive qPCR. Thick blood smears were performed according to an internationally harmonized protocol for thick smears in CHMI studies [28]. qPCR was done retrospectively using established methodologies [29] and considered positive at a parasite threshold of  $\geq 5$  parasites per mL.

#### Immunological Assays

Assessment of sporozoite invasion inhibition by volunteer serum samples was done as described previously [30, 31] and in [Supplementary Appendix 4](#). Antibody levels in citrate plasma from volunteers at baseline were measured by enzyme-linked immunosorbent assay (ELISA) to NF54 sporozoite or schizont extract. Growth inhibition was determined by invasion/growth inhibition assays (GIAs) as described in [Supplementary Appendix 4](#).

#### Sample Size Estimation and Statistical Analysis

Sample size calculation was based on the difference in prepatent period between groups. Assuming a mean time to qPCR positivity of 7.1 (standard deviation [SD], 0.8) days [32], it was

**Table 1. Demographic Characteristics of Volunteers Enrolled in The Gambia Controlled Human Malaria Infection Study**

Characteristic	High Exposure Group	Low Exposure Group	PValue
No. of participants	9	10	.752
Age, y, mean (SD)	25.7 (3.3)	22.6 (2.3)	.028
Male sex, No. (%)	9 (100)	10 (100)	
Height, cm, median (range)	177.0 (174.0–182.0)	177.0 (174.0–181.0)	.968
Weight, kg, median (range)	62.8 (59.8–80.1)	64.8 (52.8–86.7)	.490
BMI, kg/m <sup>2</sup> , median (range)	21.0 (18–26)	20.7 (18–26)	.936
Ethnicity, No. (%)			
Mandika	2 (22.2)	7 (70.0)	.043
Fula	5 (55.6)	1 (10.0)	.038
Other	2 (22.2)	2 (20.0)	.909
Residence, No. (%)			
West Coast region	7 (77.8)	7 (70.0)	.707
Upper River region	2 (22.2)	0 (0.0)	.125
Central River region	0 (0.0)	3 (30.0)	.081

Abbreviations: BMI, body mass index; SD, standard deviation.

estimated that 15 participants per cohort would be sufficient to detect a 1-day longer time to first detection of parasites by qPCR in the high-exposure group (8.1 days), with 90% power and  $\alpha = .05$ . Due to low numbers of participants presenting for screening and volunteers not meeting eligibility criteria just before study start, only 19 volunteers were enrolled. Prepatent period and parasite density at first detection by qPCR were compared between groups using the log-rank test. For the immunological analyses, differences were assessed by comparing mean values between groups or time points using either a 2-tailed Student *t* test or nonparametric equivalents. Time to patency and parasite density at first detection of infection were associated with immune responses.

## RESULTS

### Study Population

Eighty-four volunteers were screened; of these, 8 were qPCR positive during screening. Nineteen volunteers at the extremes of the immunological spectrum (Supplementary

Appendix 3) were enrolled into the study: 9 in the sero-high group and 10 in the sero-low group (Supplementary Appendix 5). Baseline characteristics are shown in Table 1. Most of the volunteers resided in the West Coast region, an area previously reported to have low transmission compared to the other regions [33]. However, malaria transmission in The Gambia is highly heterogenous with both high- and low-exposed individuals in all regions. Volunteers in the sero-high group were older than those in the sero-low group (mean age, 25.7 [SD, 3.3] years vs 22.6 [SD, 2.3] years, respectively;  $P = .028$ ).

### Parasite Kinetics and Clinical Malaria

Seventeen of the 19 volunteers (89%) developed parasitemia detected by microscopy in the first 28 days of follow-up: all individuals in the sero-low group (10/10 [100%]) and 7 (7/9 [77.8%]) in the sero-high group ( $P = .125$ ; Table 2). One of the 2 volunteers who remained microscopy negative was qPCR positive at day 18 (Figure 2A and 2B). All volunteers reported no prior or current use of antimalarial drugs and

**Table 2. Parasitological and Clinical Outcomes Following Controlled Human Malaria Infection**

Characteristic	High Exposure Group (n = 9)	Low Exposure Group (n = 10)	PValue
Subjects positive by microscopy, No. (%)	7 (77.8)	10 (100.0)	.125
Subjects positive by qPCR, No. (%)	8 (88.9)	10 (100.0)	.292
Days to parasitemia by microscopy <sup>a</sup>	14 (6.6)	13.5 (1.5)	.327
Days to parasitemia by qPCR <sup>a</sup>	11 (6.3)	9 (1.6)	.016
Days from qPCR positivity to microscopy positivity <sup>a</sup>	3 (2.6)	5 (0.5)	.156
Subjects who developed symptoms <sup>b</sup> , No. (%)	3 (33.3)	9 (90.0)	.013
Peak parasite density during study (qPCR, parasites/mL) <sup>c</sup>	3748.9 (50.6–71 264.3)	49340.3 (5186.5–205 850)	.088
AUC of parasitemia until treatment (qPCR), median (range) <sup>d</sup>	8035 (0–122 054)	34 504 (3404–120 441)	.173

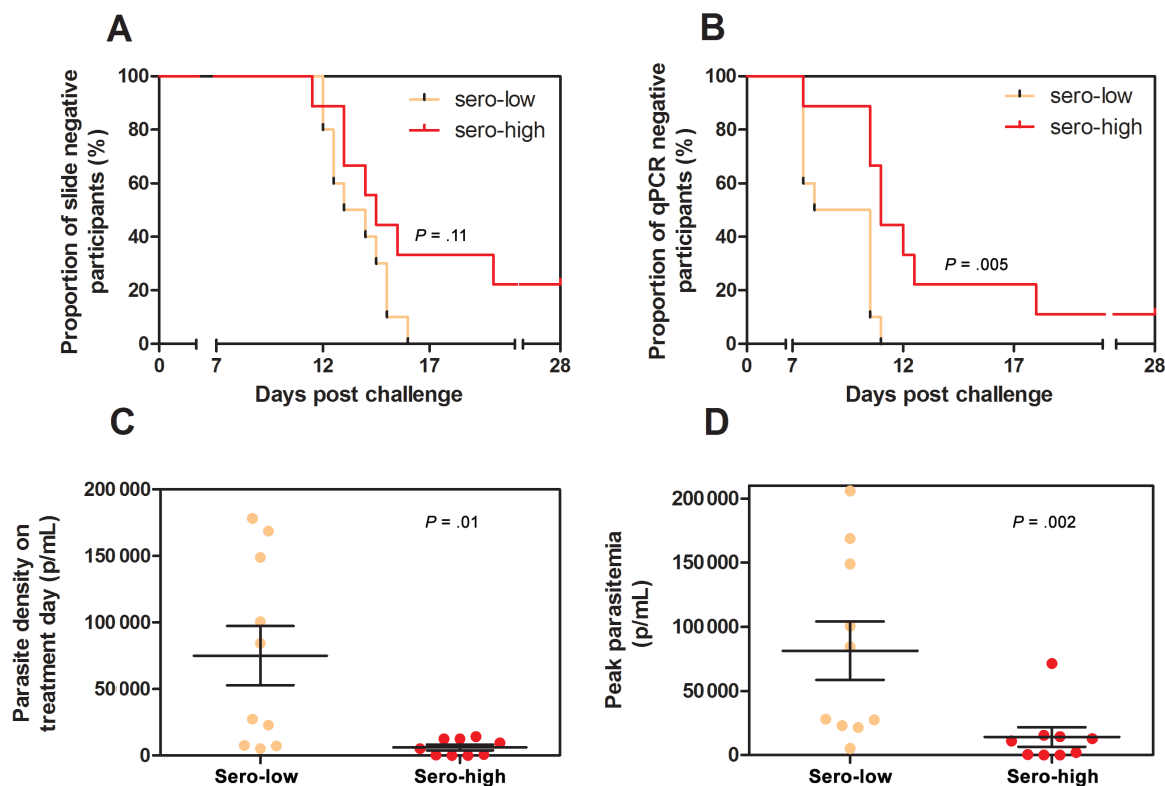
Abbreviations: AUC, area under the curve; qPCR, quantitative polymerase chain reaction (positive at  $\geq 5$  parasites/mL).

<sup>a</sup>Median (standard deviation).

<sup>b</sup>Only possibly or probably related to study.

<sup>c</sup>Geometric mean (range).

<sup>d</sup>AUC represents the total parasite exposure over time until treatment (parasite load).



**Figure 2.** Comparison of parasite kinetics between the 2 exposure groups following controlled human malaria infection. Kaplan–Meier curve for time from inoculation to parasitemia detected by thick blood smear (A) and quantitative polymerase chain reaction (qPCR) (B). Differences in parasite density by qPCR at treatment (C) and peak parasitemia (D).

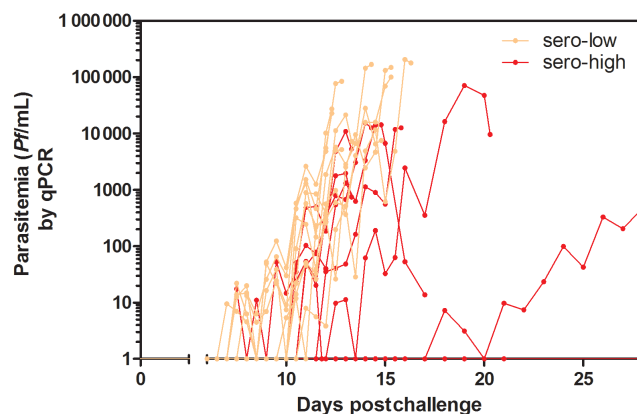
none had measurable concentrations of lumefantrine at baseline. The median prepatent period was significantly shorter in the sero-low than in the sero-high group (9.0 [SD, 1.6] days vs 11.0 [SD, 6.3] days; log-rank test,  $P = .005$ ) (Table 2, Figure 2B). Parasite density by qPCR on day of treatment was significantly higher in the sero-low than in the sero-high group ( $P = .01$ ; Figure 2C). Individual-level parasite kinetics showed faster parasite multiplication in the sero-low group (Figure 3). Parasite multiplication rates were calculated for all available 48-hour intervals ( $PMR_{48}$ ) following first detection of parasites by qPCR until treatment. The median  $PMR_{48}$  was nonsignificantly higher in the sero-low group ( $P = .143$ ) and was negatively associated with antibody titers against asexual parasite lysate ( $r = -0.5074$ ,  $P = .0376$ ) (Supplementary Appendix 6).

Participants in the sero-low group had a significantly higher probability of having clinical malaria symptoms (9/10 [90.0%]) than those in the sero-high (3/9 [33%]) group (log-rank  $P = .0008$ ; Table 2, Figure 4).

#### Safety and Tolerability of PfSPZ Challenge

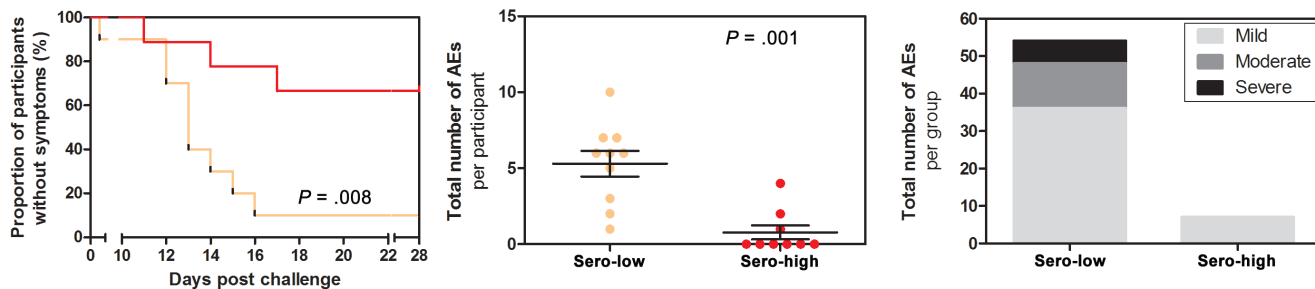
There were minimal AEs in the first 7 days after PfSPZ Challenge. Fourteen volunteers, 5 in the sero-high (55.6%) and 9 in the sero-low (90.0%) group, experienced 82 AEs, including hematological

and biochemistry abnormalities, that were possibly or probably related to malaria (Table 3). Seventy of the 82 (85.4%) AEs occurred in the sero-low group, whereas only 12 (14.6%) occurred in the sero-high group ( $P < .0001$ ). Most AEs (73/82 [89.0%]) were mild to moderate and occurred around the time parasitemia became detectable by TBS. Moderate and severe AEs were only observed in the sero-low group (Table 3, Figure 4).



**Figure 3.** Individual-level kinetics of parasitemia by quantitative polymerase chain reaction (qPCR) following controlled human malaria infection with *Plasmodium falciparum* (Pf).





**Figure 4.** Differences in clinical outcomes following controlled human malaria infection in the 2 exposure groups, showing proportion of participants without symptoms, number of adverse events (AEs) per participant, and total number of AEs per group.

was the most frequently reported AE in both the sero-high (3/12 [25%]) and sero-low (14/70 [20%]) groups. Fever was only observed in the sero-low group (5/70 [7.1%]) (Table 3). Of the 20 hematological and biochemistry abnormalities recorded, 75%

(15/20) were in the sero-low and 25.0% (5/20) were in the sero-high group ( $P = .002$ ). No serious AEs or cardiac AEs were reported, and all AEs had resolved by day 35.

**Table 3. Adverse Events Following Controlled Human Malaria Infection in the 2 Exposure Groups**

Adverse Event	Sero-high Group (n = 9)	Sero-low Group (n = 10)
Participants with any AE (including laboratory abnormalities)	5 (55.6)	9 (90.0)
Participants with grade 2 or higher AEs	2 (22.2)	8 (80.0)
Total grade 1 and 2 AEs	12	61
Headache	3 (25.0)	12 (19.7)
Fever	0	5 (8.2)
Chills	1 (8.3)	4 (6.6)
Fatigue/malaise	1 (8.3)	8 (13.1)
Myalgia	0	4 (6.6)
Arthralgia	2 (16.7)	1 (1.6)
Anorexia	0	5 (8.2)
Nausea	0	2 (3.3)
Vomiting	0	1 (1.6)
Abdominal pain	0	2 (3.3)
Dizziness	0	3 (4.9)
Diarrhea	0	1 (1.6)
Rib cage pain	0	1 (1.6)
Low platelet count	1 (8.3)	2 (3.3)
Low lymphocyte count	1 (8.3)	5 (8.2)
Low absolute neutrophil count	1 (8.3)	0
Elevated total bilirubin	0	2 (3.3)
Elevated lactate dehydrogenase	0	1 (1.6)
Elevated AST <sup>a</sup>	0	1 (1.6)
Elevated $\gamma$ -glutamyl transferase	1 (8.3)	0
Elevated sodium levels	1 (8.3)	1 (1.6)
Total grade 3 adverse events	0	9
Headache	0	2 (22.2)
Chills	0	2 (22.2)
Fatigue/malaise	0	2 (22.2)
Low lymphocyte count	0	3 (33.3)

Data are presented as No. (%).

Abbreviations: AE, adverse event; AST, aspartate aminotransferase.

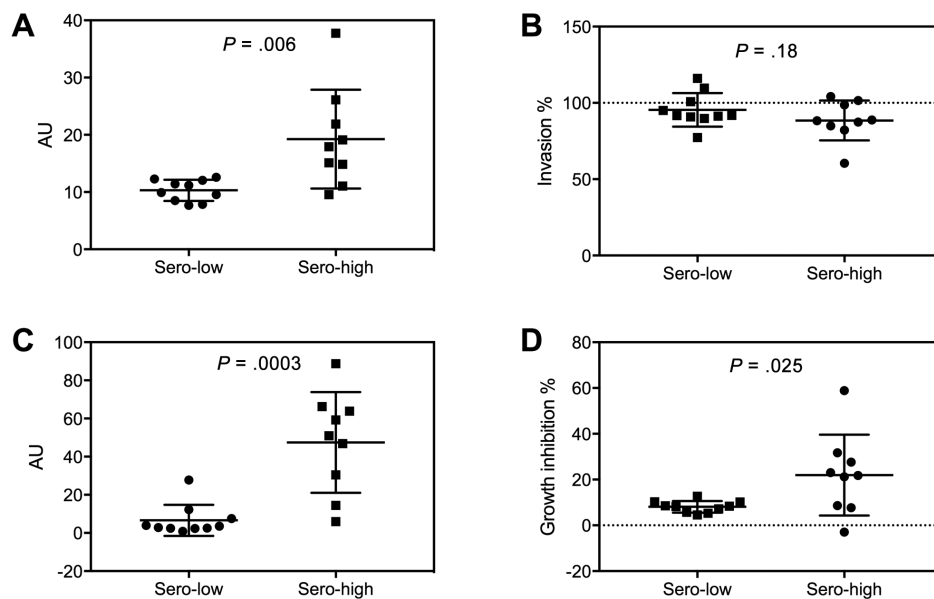
<sup>a</sup>No clinically significant elevations in alanine aminotransferase were observed.

### Humoral and Functional Immunity

Antibody recognition of sporozoites by sporozoite-binding ELISA was significantly higher in plasma of sero-high (median, 17.93 [interquartile range {IQR}, 12.95–24] arbitrary units [AU]) compared to the sero-low volunteers (median, 10.54 [IQR, 8.36–12.12] AU) ( $P = .006$ ; Figure 5A). However, the groups did not differ in their ability to block sporozoite invasion of HC04 hepatocytes, (sero-high: median 88.26% invasion [IQR, 83.52%–100.1%]; sero-low: 91.74% invasion [IQR, 90.54%–103%]) ( $P = .18$ ; Figure 5B). Invasion was indexed as a percentage relative to invasion in the presence of nonimmune serum from naive donors, where 100% meant no invasion inhibition. The presence of blood-stage antibodies, determined by schizont extract, was also significantly higher ( $P = .0003$ ) in the sero-high group (median, 50.98 [IQR, 22.46–65.07] AU; Figure 5C) than in the sero-low group (median, 3.16 [IQR, 2.43–8.71] AU). We observed indications for functional differences in blood-stage immune responses, with significantly higher GIA in the sero-high (median, 21.8% [IQR, 8.15%–29.65%]) than in the sero-low (median, 8.3% [IQR, 5.6%–10.23%]) ( $P = .025$ ; Figure 5D). Length of prepatent period correlated positively with sporozoite-binding antibody titers ( $r = 0.64$ ,  $P = .003$ ), blood-stage antibody titers ( $r = 0.48$ ,  $P = .036$ ), and blood-stage GIA activity ( $r = 0.65$ ,  $P = .003$ ) but not with sporozoite invasion inhibition ( $r = -0.29$ ,  $P = .236$ ). For individual antibody responses, Rh2.2030 ( $r = 0.5357$ ,  $P = .018$ ) and AMA-1 ( $r = 0.4959$ ,  $P = .031$ ) were the most predictive of prepatent period (Supplementary Appendix 7). Significant correlation was also seen between the different immunological responses (Supplementary Appendix 8).

### DISCUSSION

This study demonstrated the feasibility and successful implementation of CHMI with PfSPZ Challenge in The Gambia,



**Figure 5.** Antibody-mediated responses to *Plasmodium falciparum* in high- and low-exposure groups. *A*, The sero-high group had significantly higher ( $P = .006$ ) titers of antibodies to sporozoite antigens, expressed as arbitrary units (AU). *B*, There were no significant differences between groups in their ability to block sporozoite invasion of HC04 hepatocytes. *C*, Plasma from the sero-high group also had significantly higher ( $P = .0003$ ) levels of antibodies to asexual-stage antigens, also expressed as AU. *D*, Purified immunoglobulin G from the sero-high exposure group also had significantly higher growth inhibitory activity ( $P = .025$ ) against blood-stage 3D7 parasites.

increasing the capacity of conducting such studies in endemic areas: CHMI with PfSPZ Challenge has now been done in 6 African countries [5, 18–21]. A study in Gabon with PfSPZ Challenge reported that previous exposure to both *P. falciparum* and sickle cell trait impacted the rate of blood-stage infection, prepatent period, and clinical manifestations of malaria [5]. While previous studies in Kenya also associated immune responses to parasite kinetics among CHMI volunteers [34], ours is the first assessment of the effect of previous exposure to *P. falciparum* as measured by a predefined serology panel of 6 antigens on parasite kinetics, clinical symptoms, and functional immune responses. Individuals with serological evidence of higher recent and cumulative malaria exposure had a longer prepatent period, lower mean parasite density, and fewer symptoms of malaria. Whereas there was considerable variability in individual responses, the prescreening panel used to define exposure in this population correlated directly with clinical outcomes [22]. Using functional assays for preerythrocytic immunity and blood-stage immunity, this study also sheds light on the mechanisms underlying these differences. Antisporozoite responses were higher in highly exposed individuals but did not translate into responses preventing liver-stage infection in vitro while antibody responses controlling blood-stage parasite multiplication in vitro were markedly stronger in this group.

Understanding the impact of declining malaria exposure on malaria immunity is highly relevant in the context of widescale and often pronounced reductions in malaria burden in African and non-African settings [35, 36]. More direct methods for

assessing immunity are needed to quantify the clinical consequences of declined exposure. While we directly defined our cohorts based on serological markers that have been presented as indicators of recent and cumulative exposure [24, 37], several previous studies have indirectly determined malaria exposure based on self-reported clinical history of malaria episodes and long-term residence in malaria-endemic areas [5, 23] or by measuring responses to whole parasite lysate and the blood-stage antigen MSP-2 with a very long half-life [38]. In line with our findings, these studies observed a lower likelihood of parasite positivity post-CHMI in the highly exposed group [5, 23, 38]. Lell and colleagues postulated that mechanisms for the control of parasitemia included a combination of adaptive immune mechanisms such as prevention of hepatocyte infection, elimination of infected liver cells by T-cell-mediated cytotoxicity or immune mediators, and highly effective clearance of the first generation of merozoites leaving infected hepatocytes [5]. Our study directly examined differences in functional preerythrocytic and blood-stage immunity using established methodologies. Though we found no evidence for differences in inhibition of sporozoite invasion, we observed stronger parasite growth inhibition in the sero-high cohort. As volunteers were selected based on distinct immune profiles, our functional immune parameters must be interpreted with caution given challenges in disentangling functional immune responses from markers of exposure [39]. The single volunteer who remained parasite-negative by qPCR had median levels of preerythrocytic antibodies (17.93 AU), moderate HC04 invasion (104.13%;

mean, 95.41% invasion), very low levels of asexual antibodies (5.98 AU; mean, 47.42 AU), and average GIA (23% inhibition; mean, 21.94%). The striking difference in growth inhibition in our 2 cohorts suggests that functional blood-stage antibodies contributed significantly to the differences in clinical symptoms and parasite kinetics. There was a weak, negative correlation ( $r = -0.4474$ ,  $P = .0548$ ) between levels of sporozoite-binding antibodies and functional invasion-blocking activity, suggesting a minor invasion-blocking role for naturally acquired antibodies. Sporozoite-targeting antibodies in this study may be markers of exposure only or may enhance cellular immunity but lack direct invasion-blocking activity.

The systemic and laboratory AEs observed were consistent with uncomplicated malaria, with most AEs recorded at the time of positive microscopy. Severe symptoms, including chills, fatigue, malaise, and headache reported in 3 sero-low volunteers, were also consistent with uncomplicated malaria and resolved within 48 hours posttreatment. Two sero-low volunteers had grade 3 reductions in total lymphocyte count considered related to malaria and resolved by day 4 of malaria treatment. Similar declines have been reported previously [40]. This study does not allow us to extrapolate findings to other populations.

In summary, CHMI was safe and well tolerated in this population and the manifestations of malaria, although significantly different between the 2 exposure groups, were consistent with previous CHMI studies. Volunteers with high previous exposure to malaria infection were able to better control the infection as shown by the significantly lower parasite densities, less-severe symptoms, and lower incidence of symptoms associated with parasitemia.

### Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

**Author contributions.** J. A., I. J. R., G. B., T. B., C. D., R. W. S., and U. D. designed the trial, which was performed by J. A., I. J. R., E. D., and A. A. PfSPZ was generated and prepared by Y. A. for the clinical trial. Immunological assays were performed by X. Z. Y. and M. C. J. A., I. J. R., G. B., T. B., R. W. S., and U. D. provided regulatory and project support during the study. J. A., I. J. R., X. Z. Y., T. B., C. D., R. W. S., and U. D. analyzed and interpreted the data and results. J. A. and I. J. R. wrote the original draft manuscript, which was critically reviewed and approved by all authors.

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### References

- Fowkes FJ, Boeuf P, Beeson JG. Immunity to malaria in an era of declining malaria transmission. *Parasitology* **2016**; 143:139–53.
- Doolan DL, Dobaño C, Baird JK. Acquired immunity to malaria. *Clin Microbiol Rev* **2009**; 22:13–36.
- Tran TM, Li S, Doumbo S, et al. An intensive longitudinal cohort study of Malian children and adults reveals no evidence of acquired immunity to *Plasmodium falciparum* infection. *Clin Infect Dis* **2013**; 57:40–7.
- Hoffman SL, Oster CN, Plowe CV, et al. Naturally acquired antibodies to sporozoites do not prevent malaria: vaccine development implications. *Science* **1987**; 237:639–42.
- Lell B, Mordmüller B, Dejon Agobe JC, et al. Impact of sickle cell trait and naturally acquired immunity on uncomplicated malaria after controlled human malaria infection in adults in Gabon. *Am J Trop Med Hyg* **2018**; 98:508–15.
- World Health Organization. World malaria report. **2018**. Available at: <https://www.who.int/malaria/publications/world-malaria-report-2018/en/>. Accessed 18 March 2019.
- Kalayjian BC, Malhotra I, Mungai P, Holding P, King CL. Marked decline in malaria prevalence among pregnant women and their offspring from 1996 to 2010 on the south Kenyan coast. *Am J Trop Med Hyg* **2013**; 89:1129–34.
- Snow RW, Kibuchi E, Karuri SW, et al. Changing malaria prevalence on the Kenyan coast since 1974: climate, drugs and vector control. *PLoS One* **2015**; 10:e0128792.
- Mogeni P, Williams TN, Fegan G, et al. Age, spatial, and temporal variations in hospital admissions with malaria in Kilifi County, Kenya: a 25-year longitudinal observational study. *PLoS Med* **2016**; 13:e1002047.
- Griffin JT, Hollingsworth TD, Reyburn H, Drakeley CJ, Riley EM, Ghani AC. Gradual acquisition of immunity to severe malaria with increasing exposure. *Proc Biol Sci* **2015**; 282:20142657.
- Migot F, Chougnat C, Raharimalala L, Astagneau P, Lepers JP, Deloron P. Human immune responses to the *Plasmodium falciparum* ring-infected erythrocyte surface antigen (Pf155/RESA) after a decrease in malaria transmission in Madagascar. *Am J Trop Med Hyg* **1993**; 48:432–9.
- Diop F, Richard V, Diouf B, et al. Dramatic declines in seropositivity as determined with crude extracts of *Plasmodium falciparum* schizonts between 2000 and 2010 in Dielmo and Ndiop, Senegal. *Malar J* **2014**; 13:83.
- Wong J, Hamel MJ, Drakeley CJ, et al. Serological markers for monitoring historical changes in malaria transmission intensity in a highly endemic region of western Kenya, 1994–2009. *Malar J* **2014**; 13:451.
- Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can accelerate clinical malaria vaccine development. *Nat Rev Immunol* **2011**; 11:57–64.
- Stanisic DI, McCarthy JS, Good MF. Controlled human malaria infection: applications, advances, and challenges. *Infect Immun* **2018**; 86. doi:10.1128/IAI.00479-17.



16. Mordmüller B, Supan C, Sim KL, et al. Direct venous inoculation of *Plasmodium falciparum* sporozoites for controlled human malaria infection: a dose-finding trial in two centres. *Malar J* **2015**; 14:117.
17. Gómez-Pérez GP, Legarda A, Muñoz J, et al. Controlled human malaria infection by intramuscular and direct venous inoculation of cryopreserved *Plasmodium falciparum* sporozoites in malaria-naïve volunteers: effect of injection volume and dose on infectivity rates. *Malar J* **2015**; 14:306.
18. Shekalaghe S, Rutaihwa M, Billingsley PF, et al. Controlled human malaria infection of Tanzanians by intradermal injection of aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites. *Am J Trop Med Hyg* **2014**; 91:471–80.
19. Hodgson SH, Juma E, Salim A, et al. Lessons learnt from the first controlled human malaria infection study conducted in Nairobi, Kenya. *Malar J* **2015**; 14:182.
20. Jongo SA, Shekalaghe SA, Church LWP, et al. Safety, immunogenicity, and protective efficacy against controlled human malaria infection of *Plasmodium falciparum* sporozoite vaccine in Tanzanian adults. *Am J Trop Med Hyg* **2018**; 99:338–49.
21. Dejon-Agobe JC, Ateba-Ngoa U, Lalremruata A, et al. Controlled human malaria infection of healthy lifelong malaria-exposed adults to assess safety, immunogenicity and efficacy of the asexual blood stage malaria vaccine candidate GMZ2 [manuscript published online ahead of print 18 December 2018]. *Clin Infect Dis* **2018**. doi:10.1093/cid/ciy1087.
22. Lindsey Wu TH, Ssewanyana I, Oulton T, et al. Optimisation and standardisation of a multiplex immunoassay of diverse *Plasmodium falciparum* antigens to assess changes in malaria transmission using sero-epidemiology. *Wellcome Open Research* **2019**; 4.
23. Obiero JM, Shekalaghe S, Hermesen CC, et al. Impact of malaria preexposure on antiparasite cellular and humoral immune responses after controlled human malaria infection. *Infect Immun* **2015**; 83:2185–96.
24. Helb DA, Tetteh KK, Felgner PL, et al. Novel serologic biomarkers provide accurate estimates of recent *Plasmodium falciparum* exposure for individuals and communities. *Proc Natl Acad Sci U S A* **2015**; 112:E4438–47.
25. Hoffman SL, Billingsley PF, James E, et al. Development of a metabolically active, non-replicating sporozoite vaccine to prevent *Plasmodium falciparum* malaria. *Hum Vaccin* **2010**; 6:97–106.
26. Walk J, Reuling IJ, Behet MC, et al. Modest heterologous protection after *Plasmodium falciparum* sporozoite immunization: a double-blind randomized controlled clinical trial. *BMC Med* **2017**; 15:168.
27. Khalil IF, Abildrup U, Alifrangis LH, et al. Measurement of lumefantrine and its metabolite in plasma by high performance liquid chromatography with ultraviolet detection. *J Pharm Biomed Anal* **2011**; 54:168–72.
28. Laurens MB, Duncan CJ, Epstein JE, et al; Consensus Group on Design of Clinical Trials of Controlled Human Malaria Infection. A consultation on the optimization of controlled human malaria infection by mosquito bite for evaluation of candidate malaria vaccines. *Vaccine* **2012**; 30:5302–4.
29. Hermesen CC, Telgt DS, Linders EH, et al. Detection of *Plasmodium falciparum* malaria parasites in vivo by real-time quantitative PCR. *Mol Biochem Parasitol* **2001**; 118:247–51.
30. Kaushansky A, Rezakhani N, Mann H, Kappe SH. Development of a quantitative flow cytometry-based assay to assess infection by *Plasmodium falciparum* sporozoites. *Mol Biochem Parasitol* **2012**; 183:100–3.
31. Behet MC, Kurtovic L, van Gemert GJ, et al. The complement system contributes to functional antibody-mediated responses induced by immunization with *Plasmodium falciparum* malaria sporozoites. *Infect Immun* **2018**; 86. doi:10.1128/IAI.00920-17.
32. Bastiaens GJH, van Meer MPA, Scholzen A, et al. Safety, immunogenicity, and protective efficacy of intradermal immunization with aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites in volunteers under chloroquine prophylaxis: a randomized controlled trial. *Am J Trop Med Hyg* **2016**; 94:663–73.
33. Mwesigwa J, Achan J, Di Tanna GL, et al. Residual malaria transmission dynamics varies across The Gambia despite high coverage of control interventions. *PLoS One* **2017**; 12:e0187059.
34. Hodgson SH, Juma E, Salim A, et al. Evaluating controlled human malaria infection in Kenyan adults with varying degrees of prior exposure to *Plasmodium falciparum* using sporozoites administered by intramuscular injection. *Front Microbiol* **2014**; 5:686.
35. Snow RW, Marsh K. New insights into the epidemiology of malaria relevant for disease control. *Br Med Bull* **1998**; 54:293–309.
36. Rolfes MA, McCarra M, Magak NG, et al. Development of clinical immunity to malaria in highland areas of low and unstable transmission. *Am J Trop Med Hyg* **2012**; 87:806–12.
37. Drakeley CJ, Corran PH, Coleman PG, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. *Proc Natl Acad Sci U S A* **2005**; 102:5108–13.
38. Abdi AI, Hodgson SH, Muthui MK, et al. *Plasmodium falciparum* malaria parasite var gene expression is modified by host antibodies: longitudinal evidence from controlled infections of Kenyan adults with varying natural exposure. *BMC Infect Dis* **2017**; 17:585.
39. Kinyanjui SM, Bejon P, Osier FH, Bull PC, Marsh K. What you see is not what you get: implications of the brevity of antibody responses to malaria antigens and transmission heterogeneity in longitudinal studies of malaria immunity. *Malar J* **2009**; 8:242.
40. Reuling IJ, van de Schans LA, Coffeng LE, et al. A randomized feasibility trial comparing four antimalarial drug regimens to induce *Plasmodium falciparum* gametocytemia in the controlled human malaria infection model. *Elife* **2018**; 7. doi:10.7554/eLife.31549.