




STUDY PROTOCOL

Controlled Human Malaria Infection in Semi-Immune Kenyan Adults (CHMI-SIKA): a study protocol to investigate *in vivo* *Plasmodium falciparum* malaria parasite growth in the context of pre-existing immunity [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Malaria remains a major public health burden despite approval for implementation of a partially effective pre-erythrocytic malaria vaccine. There is an urgent need to accelerate development of a more effective multi-stage vaccine. Adults in malaria endemic areas may have substantial immunity provided by responses to the blood stages of malaria parasites, but field trials conducted on several blood-stage vaccines have not shown high levels of efficacy. We will use controlled human malaria infection (CHMI) studies with malaria-exposed volunteers to identify correlations between immune responses and parasite growth rates *in vivo*. Immune responses more strongly associated with control of parasite growth should be prioritized to accelerate malaria vaccine development. We aim to recruit up to 200 healthy adult volunteers from areas of differing malaria transmission in Kenya, and after confirming their health status through clinical examination and routine haematology and biochemistry, we will comprehensively characterize immunity to malaria using >100 blood-stage antigens. We will administer 3,200 aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites (PfSPZ Challenge) by direct venous inoculation. Serial quantitative polymerase chain reaction to measure parasite growth rate *in vivo* will be undertaken. Clinical and laboratory monitoring will be undertaken to ensure volunteer safety. In addition, we will also explore the perceptions and experiences of volunteers and other stakeholders in participating in a malaria volunteer infection study. Serum, plasma, peripheral blood mononuclear cells and extracted DNA will be stored to allow a comprehensive assessment of adaptive and innate host immunity. We will use CHMI in semi-immune adult volunteers to relate parasite growth outcomes with antibody responses and other markers of host immunity.

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Open Peer Review

Reviewer Status ? ✓

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	1	2
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- Salim Abdulla**, Ifakara Health Institute, Dar es Salaam, Tanzania

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

Plasmodium falciparum, controlled human malaria infection, PfSPZ Challenge, immunity, blood-stage, Kenya, parasite growth, quantitative PCR



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Abbreviations

ACT: artemisinin combination therapy; ADRB: Antibody dependent respiratory burst assay; CHMI: controlled human malaria infection; CHMI-SIKA: controlled human malaria infection in semi-immune Kenyan adults; DVI: Direct venous inoculation; GIA: Growth Inhibition Assay; OPA: Opsonic Phagocytosis Assay; PfSPZ Challenge: Aseptic, purified, cryopreserved *P. falciparum* sporozoites; qPCR: quantitative Polymerase Chain Reaction; SPZ: Sporozoites.

Introduction

Malaria remains a disease of global health importance, despite the gains made against reducing morbidity and mortality. The latest estimates of the burden of malaria indicate that 3.3 billion people are exposed with 216 million cases and over 445,000 deaths being reported, with Africa accounting for ~91% of deaths due to malaria¹. There has been encouraging progress made in some areas of Africa, but progress has now stalled². Elimination does not appear realistic in many areas with higher transmission³, and progress is threatened by insecticide and drug resistance^{4,5}. An effective vaccine strategy is required to deliver sustainable and cost-effective control⁶. Sub-unit vaccine development to date has focused on a limited pool of empirically selected candidate antigens, and field trials have not shown high levels of efficacy⁷.

Malaria vaccine development

The current lead malaria vaccine, RTS,S, a sub-unit vaccine, is based on a single pre-erythrocytic stage antigen the circumsporozoite protein (CSP). It delivers ~30% protection with waning efficacy over a few years^{8,9}. Whole *Plasmodium falciparum* (Pf) sporozoite (SPZ) vaccines have shown >90% protection against controlled human malaria infection (CHMI) and ~50% protection in the field^{10,11}. Higher vaccine efficacy would be optimal. One approach would be addition of partner antigens from the blood or transmission stages of the parasite. There are currently few subunit candidate blood-stage vaccines in clinical development: candidate vaccines in clinical efficacy trials are based on only five different antigens, and none have shown high level efficacy against their primary endpoint¹²⁻¹⁴.

A critical step in the development of a subunit blood-stage vaccine is identifying which parasite antigens to prioritise as targets for vaccine development.

Naturally acquired immunity to malaria to inform vaccine selection

One approach to understanding human immunity to malaria to date has been to identify correlates of immunity using cross-sectional surveys of children living in malaria endemic regions, identifying subsequent malaria episodes, and then linking potential correlates of host immunity to outcome¹⁵⁻¹⁸. However, the limitations of this approach include: (a) variation of exposure in the endemic population means some individuals are unexposed to infectious bites, but assessed as if they were “protected”¹⁹; (b) parasite exposures are genetically heterogeneous²⁰; (c) heterogeneity of exposure even within a single endemic setting leads to confounding effects (i.e. higher exposure is causally linked to the covariate of interest, to other effector mechanisms of immunity, and also to risk of malaria

episodes)^{21,22}; and (d) the antibody responses of greatest interest may well be those that are infrequently raised by natural exposure and are therefore not well represented in a community cohort²³.

By measuring the parasite growth rates among semi-immune adult volunteers from malaria-endemic regions taking part in controlled human malaria infection (CHMI) studies, we have the opportunity for a complementary measure of human immunity where higher levels of antibody than those generated in children can be studied, and where exposure is controlled thus avoiding the confounding factors described above.

CHMI studies in semi-immune individuals

CHMI studies in developed countries have until recently depended on exposure to mosquito bites, which place logistic demands in terms of the incubation of blood-stage cultures, feeding and infection of laboratory reared mosquitoes in sufficient numbers, which then cannot be stored. Work in malaria endemic countries can be facilitated by using cryopreserved sporozoites. Hoffman and colleagues at Sanaria have addressed various technical challenges in developing this technology, including the need to prepare large numbers of aseptic, purified and viable Pf sporozoites ((SPZ) (Sanaria(R) PfSPZ Challenge)) which can be cryopreserved and injected by syringe when required²⁴⁻³¹.

A number of studies have been conducted to date to establish the safety and efficiency of this approach^{24,25} and following a scale-up in production, CHMI studies are now possible at very much greater scale (Table 1). The direct venous inoculation (DVI) of sporozoites (PfSPZ Challenge) is the most efficient route of administration and provides a reproducible inoculum^{28,29}. Recent studies have shown that the infection rate produced by intramuscular injection of 75,000 sporozoites (PfSPZ of PfSPZ Challenge) is comparable with 3,200 PfSPZ sporozoites administered by DVI and 5 mosquito infected bites in malaria-naïve volunteers. Thus there are fewer PfSPZ sporozoites required using the DVI route of administration to achieve the same infection rates observed by the intramuscular route of administration²⁸. The DVI route has further been proven to be safe and reliable resulting in infection of all volunteers administered²⁹.

Overall, CHMI studies have been reported to be safe with only myocardial events having occurred in two naïve volunteers (from a total of over 2,000 volunteers who have undergone CHMI), one with prior cardiovascular risk factors and evidence of atherosclerotic infarction, and one without risk factors and a less clear clinical picture^{32,33}. Furthermore, no participant in CHMI has thus far developed an illness meeting criteria for severe malaria. Thus overall, administration of PfSPZ in CHMI appears to be safe. To date, there are 12 CHMI studies that have been conducted or planned in Africa involving over 480 volunteers with varying degrees of malaria exposure across 6 countries (Table 1).

Ethical considerations for CHMI studies in endemic populations

CHMI studies raise ethical issues regarding volunteer safety and as such require that careful medical supervision and mitigation of the risks of challenge. CHMI studies in endemic

Table 1. List of PfSPZ Challenge CHMI Studies in African Endemic Populations*.

Location	Study Type	Number of Volunteers	Route of Administration	Age (years)	Gender	Malaria Outcome ¹	Reference
Equatorial Guinea	Vaccine efficacy Vaccine efficacy	52 104	DVI DVI	18–35 18–45	Both Both	TBS ² TBS ²	NCT02859350 NCT03590340
Gabon	Infectivity Vaccine efficacy	20 12	DVI DVI	18–30 18–40	Both Both	TBS ² TBS ²	34 PACTR201503001038304
Gambia	Infectivity	30	DVI	18–35	Males	qPCR	NCT03496454
Kenya	Infectivity ³	28	IM	18–45	Both	TBS ²	31
Mali	Vaccine efficacy ⁴ Vaccine efficacy	62 45	DVI DVI	18–45 18–50	Both Both	TBS ² TBS ²	NCT02996695 NCT02627456
Tanzania	Infectivity ³ Vaccine efficacy Vaccine efficacy Vaccine efficacy	24 64 24 18 [^]	ID DVI DVI DVI	20–35 18–35 18–45 18–45	Males Males Both Both	TBS ⁵ TBS ² TBS ² TBS ²	30 35 NCT02613520 NCT03420053

*Current status as at 18th September 2018. ¹Primary measure for malaria treatment/diagnosis; ²Studies included qPCR data for secondary parasitaemia analysis; ³Studies included dose optimisation for PfSPZ Challenge administration; ⁴PfSPZ Challenge in the context of chemoprophylaxis using chloroquine (PfSPZ-CVac); ⁵Studies included qRT-PCR data for secondary parasitaemia analysis; and [^]PfSPZ Challenge including HIV positive individuals (N=9). DVI, direct venous inoculation; IM, intramuscular; ID, intradermal; qPCR, quantitative PCR; and TBS, thick blood smear.

populations are often carried out in in-patient settings requiring the volunteers to stay at study premises for a considerable amount of time to ensure safety and to prevent acquiring natural malaria infections^{34,35}. There has been extensive consideration of the appropriate financial compensation³⁵. Although volunteers would be motivated to participate for altruistic reasons, care should be observed to ensure that the levels of payments offered are not an undue influence which would cause the volunteers' judgement to be unduly influenced and therefore minimise the risks and discomforts involved³⁵. However, CHMI studies may require that volunteers are isolated from others, leading volunteers to incur substantial expenses and inconvenience, and these expenses must be compensated for to ensure that volunteers are not financially worse off as a result of participating. The informed consent process requires that the participant understands that they can withdraw from the study at any point with no penalty or interference^{35,36}. Nesting ethical and social empirical work within the CHMI study, we have recently shown the importance of examining participant perceptions in volunteering in CHMI studies³⁷ and aim to further explore experiences with a larger sample set and other stakeholders.

Study protocol

This study protocol has been written in accordance with the SPIRIT guidelines for reporting clinical trials. A completed SPIRIT checklist is available at the Harvard Dataverse³⁸.

Study aim and objectives

CHMI-SIKA study will investigate how the *in vivo* parasite growth rate of *Plasmodium falciparum* (Pf) is modified by pre-existing immunity measured by antibody levels to blood-stage antigens.

Primary objective

The primary objective is to measure the correlations between antibody levels to defined and well characterised malaria antigens and growth rates of *P. falciparum* in volunteers undergoing CHMI.

Secondary objectives

The secondary objectives of the CHMI-SIKA study are:

- To confirm the safety of CHMI administered by DVI in semi-immune volunteers.
- To measure parasite growth rates in semi-immune volunteers.
- To establish a sample set for the study of immunity to malaria and its effect on parasite growth following CHMI in semi-immune volunteers.
- To explore the understanding, motivations for participation, and experiences of volunteers and other stakeholders.

Study design

CHMI-SIKA is an open label, non-randomised trial involving 200 healthy volunteers aged from 18 to 45 years recruited over a period of 5 years with varying degrees of immunity from malaria endemic areas in Kenya. CHMI will comprise the direct venous inoculation of aseptic, purified, cryopreserved PfSPZ (Sanaria(R) PfSPZ Challenge). Volunteers will be enrolled for PfSPZ Challenge in groups of 20–60 volunteers per CHMI event (henceforth referred to as CHMI cohorts) at any given time. We will screen all volunteers for varying degrees of immunity, ensuring a range of volunteers with low and high antibody responses to each antigen tested. Volunteers will also be screened for significant

medical conditions before enrolling in CHMI. Blood-stage malaria infection after 6.5 days of incubation in the liver will be assayed twice daily to monitor the density of infection and anti-malarial treatment will be given either: (a) when the density of infection rises past a threshold of 500 parasites per μl (a threshold substantially lower than 2,500 parasites per μl at which clinical illness becomes more common in children in Kenya³⁹) without signs and symptoms; (b) if a volunteer develops symptoms or signs of illness and an immediate blood film examination shows any evidence of detectable malaria parasites; or (c) the volunteer reaches day 21 of monitoring, at which point CHMI will be completed (Figure 1). During the duration of CHMI, eligible volunteers will be accommodated at an in-patient setting where the volunteers will stay for the entire duration of the challenge (Figure 1). All volunteers will be provided with and required to use an ITN for the duration of the study.

Recruitment

The target volunteer population will be healthy, adult volunteers. Recruitment will target residents of Ahero in Kisumu County, Western Kenya and residents of Kilifi County, Coastal Kenya by a team of community health workers and fieldworkers respectively. Investigators will emphasize that participation in the study is voluntary. Participant information sheets will be given to interested volunteers. Individuals who feel that the trial is potentially appropriate for them will be invited to attend a formal screening visit with a study clinician. Recruitment in Kilifi will target residents within Kilifi North (Ngerenya) and Kilifi South (Pingilikani and Junju) sub-locations of Kilifi County, where malaria transmission is low to moderate⁴⁰. Recruitment in Kisumu will target residents of Ahero where transmission is high⁴¹.

In Kilifi, community wide meetings to inform potential volunteers about the study will be conducted in the sub-locations followed by meetings with smaller groups of potential volunteers who are interested in participating by fieldworkers. In Ahero,

community health workers will conduct meetings with smaller groups of potential volunteers. Volunteers from Ahero will have to be fluent in Kiswahili and/or English, as they will be asked to travel to Kilifi for the challenge event.

Recruitment for the social science component will involve only volunteers who are recruited, consented, and eligible to be enrolled for PfSPZ Challenge administration. Following recruitment to the CHMI study, volunteers will undergo a separate consenting process³⁷ to take part in the social science arm of the study. In addition, relevant stakeholders involved in the study including study clinical and research staff, community representatives and/or field workers will be recruited and consented for participation in this aspect of the study. All volunteers in this part of the study will be recruited after a process of information giving about this component of the study. All consent forms are available to view on Harvard Dataverse³⁸.

Screening

All potential volunteers will have a screening visit either at the clinical facility in Ahero (Ahero Clinical Trials Unit, ACTU) or in Kilifi at the Kenya Medical Research Institute Wellcome Trust Research Programme (KEMRI-WTRP) which may take place up to 120 days prior to enrolment into a CHMI cohort. The screening visit will involve assessment of malaria past exposure using both laboratory tests and history. Informed consent will be taken before screening. If consent is obtained, the screening procedures indicated in the schedule of procedures (Table 2) will be undertaken. Abnormal clinical findings from the medical history, clinical assessment or blood tests at any point in the study will be assessed. If a test is deemed clinically significant it may be repeated to confirm the result. If an abnormal finding is deemed to be clinically significant, the volunteer will be informed and referral to an appropriate medical centre arranged with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

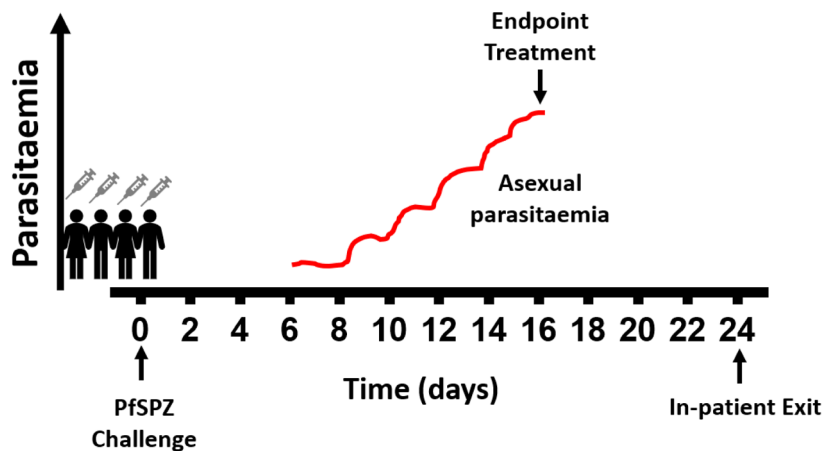


Figure 1. Schematic of CHMI PfSPZ challenge study profile.

Table 2. Schedule of Study Procedures.

Timeline (days in relation to challenge)	Screening	C-1	C	C+1 – C+24	C+35
Window (days)	-120 to -3	0	0	0	±5
ICF and ICF evaluation	X				
Medical History	X				
Clinical Assessment	X	X	X	X	X
Urinalysis	X				
Urinary β -hCG	X	X		X	
ECG	X				
Measurement of Height and Weight		X			
Administration of PfSPZ Challenge			X		
Local and Systemic events reviewed		X	X	X	X
Anti-malarial treatment directly observed				X	

C, PfSPZ Challenge; C-1, day before PfSPZ challenge; C+1, day 1 after PfSPZ challenge; β -hCG, β -human chorionic gonadotrophin; ECG, electrocardiogram. Clinical assessment will be based on tests conducted from blood samples collected.

Selection of volunteers

Inclusion criteria

- Healthy adults aged 18 to 45 years.
- Able and willing (in the Investigator's opinion) to comply with all study requirements.
- Informed consent.
- Use of effective method of contraception for the duration of study (women only). We will ask the female volunteers to come with their family planning records to verify. Effective contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly, in accordance with the product label. Examples of these include: combined oral contraceptives; injectable progestogen; implants of etenogestrel or levonorgestrel; intrauterine device or intrauterine system; male partner sterilisation at least 6 months prior to the female subject's entry into the study, and the relationship is monogamous; male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository); and male condom combined with a female diaphragm, either with or without a vaginal spermicide (foam, gel, film, cream, or suppository).

Exclusion criteria

Any of the following constitutes exclusion criterion:

- Use of systemic antibiotics with known antimalarial activity within 30 days of administration of PfSPZ challenge (e.g. trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones and azithromycin).

- Receipt of an investigational product in the 30 days preceding enrolment, or planned receipt during the study period.
- Current participation in another clinical trial or recent participation within 12 weeks of enrolment.
- Prior receipt of an investigational malaria vaccine.
- Previous receipt of malaria sporozoites (PfSPZ) as part of the malaria challenge study.
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed).
- Use of immunoglobulins or blood products within 3 months prior to enrolment.
- Any serious medical condition reported or identified during screening that increases the risk of CHMI.
- Any clinically significant abnormal finding on biochemistry or haematology blood tests, urinalysis or clinical examination.
- Women only; pregnancy, or an intention to become pregnant during the duration of the study.
- Confirmed parasite positive by PCR a day before challenge i.e. at C-1.

Exclusion criterion on day of PfSPZ Challenge administration:

- Acute disease, defined as moderate or severe illness with or without fever (temperature $>37.5^{\circ}\text{C}$).

Enrolment

We will enrol up to a total of 200 volunteers in CHMI. All volunteers will give written informed consent before being enrolled, after having been informed of the nature of the study, the potential risks and their obligations. We will conduct the CHMI studies in groups of 20 to 60 volunteers per cohort as guided by logistic and operational considerations.

Consent. All volunteers will sign and date the informed consent form before any study specific procedures are performed. The clinician or investigator will have a checklist for them to discuss with the potential volunteer aimed to assess their understanding of the study. The information sheet will be made available to the volunteer at least 24 hours prior to the screening visit. All informed consent documents will be translated into local languages (Dholuo, Kiswahili and Giriama). Particular points to be covered will be:

- Participation in the study is entirely voluntary.
- Declining to participate involves no penalty or loss of medical benefits.
- A volunteer may withdraw from the study at any time.
- A volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved.
- There is no direct benefit from participating. The benefits will be realized in the long-term for the community by contributing towards the development of a malaria vaccine.
- Volunteers will be compensated for travel, time and inconvenience of participating

Withdrawal criteria. In accordance with the principles of the current revision of the Declaration of Helsinki (updated 2008) and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being. In addition, the volunteer may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening)
- Significant protocol deviation
- Volunteer non-compliance with study requirements

Managing withdrawals. The reason for withdrawal will be recorded in the [study case report form](#) (CRF)³⁸. If withdrawal is due to an adverse event (AE), appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilised or a non-study related causality has been assigned. If a volunteer withdraws/is withdrawn from the study after receiving PfSPZ Challenge but before reaching the criterion for malaria diagnosis, a complete, appropriate dose of the recommended anti-malarials will

be provided and directly observed by the study clinical team. The importance of taking this medication will be stressed to the volunteer. If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used and stored. Data from volunteers withdrawn from the study before fulfilling the criterion for malaria diagnosis will be excluded from the analysis of results relating to the study's primary objective. Data from volunteers withdrawn from the study after fulfilling the criterion for malaria diagnosis will be included in analysis of results relating to the study's primary objective.

Study procedures

Procedures will be performed at the time points indicated in the schedule of procedures in [Table 2](#). Additional procedures or laboratory tests may be performed at the discretion of the investigators if clinically necessary (e.g. urine microscopy in the event of positive urinalysis). Observations including pulse rate, blood pressure, respiratory rate and temperature will be measured at the time points indicated in the schedule of procedures. In addition, for blood tests, blood will be drawn at various time points ([Table 2](#) and [Table 3](#)) and the following laboratory assays performed: haematology (complete blood count (CBC); biochemistry (assessing sodium, potassium, urea, creatinine, albumin, ALT and bilirubin); diagnostic serology for HIV antibodies, Hepatitis B and Hepatitis C; immunological assays of prior exposure to malaria including antibody and cell-mediated immunity; diagnostic malaria tests being primarily qPCR for *P. falciparum* DNA and microscopy, and for detection of parasite sexual stages using a reverse transcriptase PCR assay; and DNA Genotyping PCR for red blood cell polymorphisms including but not limited to sickle cell trait, alpha-thalassemia, Dantu mutation of glycoporphin A, and others that might affect susceptibility or resistance to malaria⁴². Urinalysis will be done for the presence of clinically significant proteinuria, glucosuria or haematuria at screening and at various follow-up time points.

Table 3. Schedule of blood samples and volumes (in ml) for Screening to day before PfSPZ Challenge.

Variable	Screening	Repeat PCR check (where applicable)	C-1
PCR [§]	4	4	4
FBC	1		1
Biochemistry*	2		2
Serology (HIV and Hepatitis B) [^]	1		
Whole blood in RNA stabilizing buffer [#]	2		2
Plasma/PBMCs ⁺	10		50
Volume (ml)	20	4	59
Cumulative Total (ml)	20	24	83

C-1= day before PfSPZ challenge; [§]Includes screening for sickle cell trait, alpha-thalassaemia and other red blood cell polymorphisms and for gametocyte detection; *Biochemistry will include sodium, potassium, urea, creatinine, albumin, ALT and bilirubin; [^]for assessing antibody responses; [#]for transcriptome analysis; ⁺for immune responses.

Urine will also be tested for β -human chorionic gonadotropin (β -hCG) in female volunteers at screening, prior to administration of PfSPZ challenge and prior to start of anti-malarial medication. Electrocardiograms (ECGs) will be performed at screening and examined by a clinically qualified investigator for evidence of heart disease.

For HIV diagnostic testing, volunteers will have information provided prior to HIV test counselling. HIV sero-status will be established using the standard rapid diagnostic kits in the lab as per the testing algorithm used by the Kenyan Ministry of Health. Those diagnosed as HIV antibody-positive will be referred to an appropriate health centre for further counselling and treatment. To maintain the confidentiality of those volunteers infected with HIV, we will make it clear that during screening one can be excluded due to a range of conditions (not just HIV), as well as abnormal laboratory results.

Malaria infection prior to PfSPZ Challenge. In order to prevent pre-existing malaria infection from interfering with the study, eligible volunteers who are found to be PCR-positive for malaria infection at screening will be treated with 7 days of artesunate (this drug was chosen to avoid other drugs with long half-lives that might interfere with subsequent CHMI), and the volunteer will then be screened again by PCR to confirm that they are negative in the week before being included in CHMI. In the case where a time lag of 10 days or more occurs between screening and challenge, all screened volunteers will be treated with the 7-day artesunate observed dose and a repeat PCR screen undertaken at least three days before CHMI (C-3). CHMI will be undertaken during the dry seasons to avoid high rates of malaria re-infection leading to volunteers being ineligible.

Preparation and administration of PfSPZ Challenge. For preparation of PfSPZ Challenge, immediately prior to use, PfSPZ Challenge in cryovials will be thawed individually by partial submersion of the vials for 30 seconds in a $37\pm 1^\circ\text{C}$ water bath. Designated, trained study staff will then prepare, dilute and dispense PfSPZ Challenge to clinical staff. Aliquots of the diluents phosphate buffered saline (PBS) and 25% human serum albumin (HSA) will be provided to the clinical sites by Sanaria Inc. PfSPZ Challenge will be administered using a needle and syringe by DVI.

During administration of PfSPZ Challenge, advanced life support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. Volunteers will be observed for 1 hour after injection at the study clinic before returning to the in-patient setting located ~2.5km from the KEMRI Wellcome Trust Research Programme (KEMRI-KWTRP) at the local university (i.e. Pwani University). Following PfSPZ Challenge, volunteers will be resident as in-patients until the treatment criteria are met or the withdraw/are withdrawn. The injection sites will be covered with a sterile dressing. The sterile dressing will be removed no earlier than 1 hour after inoculation. Any unsolicited adverse events or serious adverse events (SAE) will be recorded. (See Safety Monitoring below).

We will inject 3,200 parasites DVI. This will be done by a trained clinician with observation of the injection technique from personnel experienced in PfSPZ injection. The dose and route of administration of PfSPZ Challenge to be used in this trial have been chosen to maximise the likelihood of successful infection with malaria and are based on data from previous trials of PfSPZ Challenge in malaria-naïve individuals and in malaria endemic regions (see Introduction).

Monitoring after PfSPZ Challenge. A day before (C-1) administration of PfSPZ Challenge, all eligible volunteers will be asked to report to the study clinic to be re-assessed. All volunteers will be required to be enrolled at an in-patient setting near the study clinic in Kilifi until completion of endpoint anti-malarial treatment. Assessment of any new medical conditions or symptoms that have arisen since screening will be performed. Clinical assessments (including measurement of height and weight), urinary β -hCG and blood tests will be undertaken according to [Table 3](#) and [Table 4](#). Results of blood tests taken at this visit will be made available and reviewed prior to PfSPZ Challenge administration.

On the day of PfSPZ Challenge administration, all volunteers will have clinical assessments performed prior to CHMI and review of all C-1 results. If withdrawal criteria are met this will be dealt with as described above. An hour after PfSPZ Challenge administration, volunteers will be monitored and any AEs occurring will be documented (solicited and unsolicited [Table 5](#)).

The liver stage of malaria infection is asymptomatic and lasts 6 days. A blood sample will only be collected on day 5 post-PfSPZ Challenge (C+5), where a venous blood sample will be taken for assessment of liver stage immunity ([Table 4](#)). During this period volunteers will be required to be resident at the in-patient setting and will have access to a study clinician and nurse in case of any symptoms. Between day one and four (C+1 – C+4), volunteers will be asked for their consent to participate in the social science component of the study. Those willing to participate will be asked to fill out a questionnaire regarding their expectations of the study and regarding the information they have received. In addition, 20–25 volunteers will be selected based on age, religion, and gender (to get a diversity of volunteers) and requested to participate in in-depth interviews and focus group discussions. All questionnaires are available on Harvard Dataverse³⁸.

During blood stage infection, between days 7 and 21 (C+7 to C+21), there will be daily review of the volunteers. At each review the following will be carried out:

- Clinical assessment.
- Volunteers will be questioned as to whether they have experienced any symptoms of malaria.
- Venous blood sampling will be performed as per schedule of attendance ([Table 4](#)).
- Severity of symptoms will be assessed using grading criterion summarised in [Table 6](#).

Table 4. Schedule of blood samples from challenge to challenge +35 days.

Days after challenge	5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	16	17	18	19	20	21	Diag ⁶	+24hr	+48hr	+72hr	C+35	
PCR		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
FBC		1														1								1	1			1	1	
Biochemistry ^{4,5}						2																		2	2					
Plasma/PBMCs ⁵	30	30				30										30								30	30					50
RNA analysis	2	2				2										2								2	2					
Parasite Typing																								2	2					
Vol (ml)	32	37	4	4	4	38	4	4	4	4	4	4	4	4	4	37	4	4	4	4	4	4	4	41	41	4	4	5	51	
Cumulative ⁶ Total (ml)	115	152	156	160	164	202	206	210	214	218	222	226	230	234	238	275	279	283	287	291	295	299	303	344	-	348	352	357	412	

Only one blood samples will be drawn between day of PISPZ Challenge and C+6 (C+5). ⁴Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, ALT & Bilirubin. ⁵Blood will be drawn for biochemistry on +9, C+21 and day of diagnosis. ⁶Blood will be taken for plasma and/or PBMCs on +5, +7, +9, +14, +21, and day of diagnosis and then not again until C+35. ⁶Blood sample will not be taken at diagnosis if a sample has already been taken on the same day. ⁷The cumulative total includes pre-challenge blood volumes indicated in Table 3 (i.e. 83ml + 32 ml to give first cumulative total of 115 ml).

Table 5. Solicited adverse events related to malaria infection.

Adverse events	
Physical Signs	Fever
	Hypotension
	Tachycardia
Symptoms	Feverishness
	Chills
	Rigor
	Sweating
	Headache
	Anorexia
	Nausea
	Vomiting
	Myalgia
	Arthralgia
	Low Back Pain
Laboratory Abnormalities	Fatigue
	Lymphopenia
	Thrombocytopenia

Table 6. Severity grading for adverse events.

Grading	Definition
Grade 0	None
Grade 1	Mild: Transient or mild limitation in activity (<48 hours); no medical intervention/therapy required
Grade 2	Moderate: Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
Grade 3	Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalisation possible

Venous blood samples will be taken for qPCR for *P. falciparum* twice per day from days C+7 to C+14, and then once per day from C+15 to C+21. qPCR results will be processed within 6 hours of collection for the morning tests and within 18 hours for the evening tests. Endpoint anti-malarial treatment will be given based on qPCR results at a threshold of 500 parasites per μl (see study treatment below). A sensitive high-volume qPCR assay will be used for detection and any production of gametocytes using a reverse transcriptase PCR assay.

Following diagnosis, endpoint, volunteers will continue to be reviewed and have clinical observations performed once a day post-diagnosis. If qPCR results from blood taken at 24, 48 and 72 hours post-diagnosis are negative for parasites and the patient has no symptoms or mild, resolving symptoms, then the volunteer will be able to leave the in-patient setting and not reviewed again until C+35. If further blood samples are positive or symptoms are persistent then a medical assessment will be

conducted, and further investigation or treatment planned according to the findings. On the last day of anti-malaria treatment, a blood sample will be drawn for complete blood count (Table 4) for safety monitoring.

All volunteers will be required to attend a review clinic visit 35 days after challenge administration. Clinical assessments will be performed, and AEs assessed. Venous blood samples will be collected (Table 4). In addition, a questionnaire will also be given during the C+35 visit to explore the perceptions of the volunteers regarding their experience in the study. For the in-depth interviews and discussions, 20–25 volunteers who were previously selected (during C+1 to C+6 above) will be requested to participate in more in-depth discussions.

Study treatment

Clinical reviews. All clinical care and procedures will be undertaken by a qualified nurse and clinician trained in the

study procedures. Following administration of PfSPZ challenge volunteers will remain at the in-patient setting until the endpoint treatment completion. Clinically qualified staff will be available at all times, and standard operating procedures (SOPs) will be established for out-of-hours assessment and management of symptoms. Volunteers and staff at the site will have contact numbers of clinically qualified investigators who in turn can consult a senior clinician at any time during CHMI.

The volunteers will be monitored by clinical staff with experience of managing clinical *P. falciparum* infection. Resuscitation equipment and anti-malarial drugs will be available at all times. The strain of parasite Pf, NF54 used for CHMI is known to be sensitive to chloroquine, artemether-lumefantrine (AL), atovaquone/proguanil and sulphadoxine-pyrimethamine (SP). These treatments are all known to be effective for uncomplicated malaria. A full treatment course will be given to all volunteers reaching the end of CHMI.

Malaria management. All volunteers will be treated with a full course of artemether-lumefantrine as endpoint anti-malaria treatment. Doses of treatment will be directly observed. The infecting parasites are known to be fully sensitive to artemether-lumefantrine. Endpoint anti-malarial treatment will be given based on qPCR results at a threshold of 500 parasites per μl . In addition, if a volunteer develops symptoms or signs of malaria then a rapid diagnostic test will be done and a sample taken for an immediate blood film examination to be conducted and results relayed immediately on availability. If any parasites are seen on the blood film then anti-malarials will be given. Blood films will be prepared by an experienced microscopist and 100 high power fields of both thick and thin films will be examined for parasites before the film is declared negative. The presence of a parasite will be confirmed by a second microscopist before the film is considered positive. Additionally, if the clinical investigators have concerns regarding the clinical condition of any volunteer they may advise treatment with anti-malarials irrespective of the results of PCR or microscopy. When a case of malaria is diagnosed, each subject will have a clinical evaluation by one of the investigators (a clinician) with appropriate history and clinical examination.

Volunteers who remain undiagnosed with malaria at C+21 will start a treatment course of artemether-lumefantrine at this time point. With this close observation we do not anticipate any serious illness developing. However, if in the opinion of a clinical investigator a volunteer shows signs that indicate the need for in-patient care then admission will be organized an appropriate hospital. If intensive care facilities are required or may be required, then a referral will be made to an appropriately equipped facility.

If a patient is unable to tolerate an oral anti-malarial, they will be treated with parenteral artesunate until they are able to take oral medication. If a volunteer withdraws/is withdrawn from the study after receiving PfSPZ Challenge but before reaching the criterion for malaria diagnosis, a complete, appropriate, curative course of anti-malarial therapy must be completed. The

importance of this will be emphasised to volunteers at screening. If a volunteer develops a contraindication to artemether-lumefantrine or is unable to tolerate artemether-lumefantrine, oral chloroquine or SP may be prescribed as an alternative treatment for malaria. The strain of *P. falciparum* used is known to be sensitive to chloroquine, atovaquone/proguanil, SP, and artemether-lumefantrine. These drugs will be used in accordance with the manufacturer's instructions and the Government of Kenya treatment guidelines.

Safety monitoring

Safety oversight will be the responsibility of the investigators and the Data Safety Monitoring Committee (DSMC) that will be convened.

DSMC. A DSMC will be convened on behalf of the sponsor and will consist of 5 individuals who cover clinical and statistical expertise including at least 2 DSMC members based in Kenya. In addition, a member from Sanaria and one from Clinical Trials Facility in Kilifi will be available for the DSMC meetings to provide information and clarifications but will not be voting members on the DSMC and will not take part in the closed DSMC session. The DSMC will receive reports of all SAEs and suspected unexpected serious adverse reactions (SUSARs) as well as volunteers lost to follow up during CHMI. The DSMC will be empowered to stop some or all trial procedures by recommendation to the Sponsor. If such a recommendation is made, then research ethics committees will be informed within 3 working days of the recommendation. The DSMC Charter will be drawn before participant enrolment. Regular monitoring will be performed according to International Council for Harmonisation (ICH) good clinical practice (GCP) and a monitoring plan. Monitors will check whether the clinical trial is conducted, and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The site team led by the PI will be responsible for local submissions to the research ethics committees and all the staff will have good clinical practice training prior to study start.

Safety measures for PfSPZ Challenge. Volunteer safety is of paramount importance. The following measures will be in place to safeguard volunteer safety:

- All volunteers will be asked to provide details of an emergency contact person who may be contacted if the volunteer cannot be contacted or located following CHMI and before treatment.
- All doses of artemether-lumefantrine will be observed by the study team. (For volunteers taking other anti-malarials, at least half of all doses will be observed).
- Volunteers will be counselled to contact the study team for review if they develop fever or other symptoms of malaria in the 6 months following the challenge.

Adverse events (AEs). AEs will be documented in individual CRFs for each volunteer. They will be recorded under two headings; solicited and unsolicited. Any unforeseen and unavoidable deviations from the study protocol will be documented and

filed in a protocol deviation folder, with explanation. Case report forms will be kept securely. The severity of AEs will be graded using the scale provided in [Table 6](#). Events include:

1. Adverse events: Any untoward medical occurrence in a patient or clinical investigation subject occurring in any phase of the clinical study whether or not considered related to the vaccine. This includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, or drug interactions. Anticipated day-to-day fluctuations of pre-existing conditions, including the disease under study, that do not represent a clinically significant exacerbation will not be considered AEs. Discrete episodes of chronic conditions occurring during a study period will be reported as adverse events to assess changes in frequency or severity.

Unsolicited adverse events will be documented in terms of a medical diagnosis/diagnoses. When this is not possible, the AE will be documented in terms of signs and symptoms observed by the investigator or reported by the subject. Pre-existing conditions or signs and/or symptoms (including any which are not recognised at study entry but are recognised during the study period) present in a subject prior to the start of the study will be recorded on the medical history form within the subject's CRF.

2. SAEs: A SAE will be any untoward medical occurrence that at any time:
 - results in death,
 - is life-threatening,

Note: The term “life-threatening” in the definition of “serious” refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity, or
- results in a congenital anomaly/birth defect.

Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These will also usually be considered serious. SAEs related with drug treatment will not be reported to a sponsor, since the drugs are all licensed, but we will undertake expedited reporting to the DSMC and the ethics committees.

3. Suspected unexpected serious adverse reactions (SUSAR): An adverse reaction, the nature or severity

of which is not anticipated based on the applicable product information is considered as an unexpected adverse drug reaction. Where the adverse reaction is also considered to have a possible, probable or definite relationship with the drugs given, and also meets the criteria for a serious adverse reaction, it is termed a SUSAR. These events are subject to expedited reporting as for SAEs.

Reporting SAEs and unexpected AEs. SAEs will be reported within 24 hours of their identification by study staff to the research ethics committees and DSMC. SUSARs will be reported within 1 working day to the same parties. If any volunteers are lost to follow up during CHMI without having completed a course of anti-malarials this will also be reported within 1 working day. The DSMC will be empowered to stop recruitment or study procedures if they believe it is required to protect the safety of volunteers. Adverse events likely to be related to CHMI, whether serious or not, which persist at the end of the trial will be followed up by the investigator until their resolution or stabilisation. The outcome will be assessed as: recovered/resolved; not recovered/not resolved; recovering/resolving; recovered with sequelae/resolved with sequelae; and fatal (SAEs only).

Assessment of immunity to malaria

Immune responses, antibody and cell-mediated, will be assayed to determine signatures of immunity using plasma and peripheral blood mononuclear cells (PBMCs). For assessment of antibody immunological responses, techniques will include protein microarrays, antibody-dependent assays of functional immunity such as (GIAs)⁴³⁻⁴⁵, opsonic phagocytosis assays (OPA)⁴⁶, and antibody dependent respiratory burst (ADRB)^{44,45,47}.

A detailed analysis of cellular immune responses in PBMC, plasma and host transcriptomics will be undertaken and include the following: flow cytometry to determine cellular correlates of immunity; determination of the evolution of the T and B cell response at the transcriptomic and cellular levels; RNA sequencing and multiphoton microscopy to study the impact of exposure to parasites on dendritic cells; and determination of the role of antibody-mediated *P. falciparum* sporozoite inhibition. In addition, the parasite transcriptomes will be analysed as well as assessment of parasite isolates on the day of diagnosis to ensure that the signs and symptoms of malaria observed in the volunteers are not due to community-acquired infections but the infecting parasite strain, NF54.

Sample use and storage. This study will provide a platform for further studies to be conducted using the samples to be collected and stored. Additional ethics approval will be obtained for sub-analyses if different from primary and secondary objectives. The sample set is likely to be an important international resource for testing responses to new candidate antigens for vaccine development, and therefore we will need to store samples to allow further collaborations in the future. These collaborations will be supported if they are consistent with the

protocol and as approved by the relevant research ethics committee. Testing outside the permission granted in this protocol will only be supported following an amendment or a new protocol. The samples collected will be stored in the KEMRI-Wellcome Trust Research Programme Kilifi Repository.

Data management

The principal investigator (PI) will have overall responsibility for ensuring management of the data. A designee to the PI will be responsible for receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. Responsibility for this may be delegated to the study data management team. The data will be entered into the subjects' paper [screening CRF](#) and [study CRF](#)³⁸. Data will be subsequently transferred to an electronic database for analysis. If any changes to the study are necessary during the study a formal amendment will be presented to the sponsor prior to submission to the relevant ethical and regulatory agencies for approval unless to eliminate an immediate hazard(s) to study participant without prior ethics approval. Any unforeseen and unavoidable deviations from the protocol will be documented and filed in as a protocol deviation in the Trial Master File, with explanation. A protocol deviation will be any failure to adhere to the defined procedures or treatment plans outlined in the protocol version previously approved by the relevant local research ethics committee. A protocol violation is any planned or inadvertent changes that may impact safety of study participants, affect integrity of the study data and/or affect study participants willingness to participate in the study previously approved by the relevant local research ethics committee. Both deviations and violations will be reported to the relevant local research ethics committee within 10 working days of the deviation/violation. The investigators will formulate a management plan to avoid the occurrence of a similar deviation in future.

Data capture methods and archiving. Data capture will be primarily via an offline database for the scheduled visits through use of computers and laptops. These data will then be transferred to an eCRF on Open Clinica. Paper source documents will be used to capture data for the screening visits, unscheduled clinic visits, laboratory and other investigational results. Data on scheduled visits may still be captured on paper source documents if electronic methods are not available. Immunological and PCR data will be transferred to an electronic database for analysis without any volunteer identifier apart from the unique volunteer number. For the social science component, the interviews and discussions will be recorded^{37,38}. For archiving, the investigator will keep the consent forms and trial master file for at least 5 years after the completion or discontinuation of the trial. The anonymized electronic databases will be maintained beyond this period.

Data sharing, reporting, and dissemination. We will make the information on antigen prioritization publicly available and open access as rapidly as possible to provide the widest possible benefit of the study. We will feedback individual results with clinical relevance to volunteers in real-time. Summaries of the outcomes of the trial will be provided during community

meetings in the areas from which volunteers are recruited. It is not anticipated that substantial information in this form will be available until at least the third year of recruiting, and this will be made clear during initial meetings to avoid unrealistic expectations regarding the rapidity of feedback.

Statistics

Sample size. Sample size calculations were done considering power of the multivariable model using the `powerreg` facility from STATA 13, assuming an r^2 value of 0.3 for the combined model (i.e. including all variables) indicates 80% power to detect a single variable accounting for 0.15 of the variability in growth rates (after adjusting for other variables) on examining 200 volunteers when 50 variables are included in the model.

Statistical and analytical plans. Our analyses of these data will test the following hypotheses: (1) that naturally acquired immunity to malaria depends on responses to specific identifiable antigens; (2) that there are specific thresholds above which immunological responses control parasite growth; and (3) the overall intensity and/or breadth of antibody responses to parasite antigens are associated with immunity. These will be tested using generalized linear models using parasite growth patterns to derive the outcome variable. The parasite growth outcomes will be categorized by analysis of the PCR data and published prior to development of the statistical analysis for immune correlates.

For the qualitative analysis, the recorded interviews will be transcribed and translated where necessary. The data will be organized and managed using NVIVO 10, a qualitative data management and analysis software. The data will be analysed using a thematic content analysis, which will allow for categorization of the recurrent and common themes and will allow for the presentation of the key elements of the volunteers' accounts.

Ethical compliance

Study registration. The study has been registered with the ClinicalTrials.gov registry ([NCT02739763](#)).

Ethical compliance. Ethical approval has been granted by the institutional research ethics committee, Kenya Medical Research Institute Scientific and Ethics Review Unit (KEMRI/SERU/CGMR-C/029/3190) and by the sponsor of the study (University of Oxford) through the Oxford Tropical Research Ethics Committee (OxTREC, 2–16). The regulatory authority granted exemption of review for the study under the 2011 guidelines (Pharmacy and Poisons Board, PPB/ECCT/Misc/2015(61)).

Confidentiality and data protection. The clinical records will be kept in locked cabinets in the clinical trials facility. All immunological and qPCR data will be kept in anonymized databases linked by the study number to clinical data. For the qualitative social science component, all the recordings will be destroyed after the study has been completed. The data will be stored in password-protected computers and the hard copy documents will be stored in lockable cabinets. The interviews and discussions will be conducted in spaces that ensure privacy and confidentiality of the information provided by the volunteers

as well as provide an environment that volunteers can freely express their opinions

Community engagement. Each site, Ahero CTU in Ahero and KWTRP in Kilifi will use existing community engagement strategies to inform communities about the study. For Kilifi, this will be the first CHMI study to be performed in the area and therefore there will be concerted effort together with the long-established community liaison group (CLG) to ensure that information giving, and community engagement activities are extensively carried out. A community engagement plan specific for the study will be developed between the CLG team and the investigators. Community meetings in areas of participant recruitment, meetings with chiefs, sub-chiefs, community representatives, and the Department of Health, Kilifi County will be organized to inform them about the study. Community engagement will continue throughout the study period of five years, collecting and responding to concerns from the community about the study through the CLG team.

Publication policy. Results will be published in an open-access journal. Anonymized data on PCR values and immunological data will be made available with these publications. The scientific and intellectual contributions of all persons involved in the research will be appropriately acknowledged in all publications and presentations arising from the work.

Study status. Ethical approval for CHMI-SIKA was granted in March 2016 and the study started enrolment in August 2016. To date (30th October 2018) we have enrolled 161 volunteers with two DSMC meetings. The foregoing protocol is written in the future tense reflecting that work is ongoing.

Discussion

Malaria transmission is an ongoing public health problem in Africa. There is an urgent need to accelerate the development of a more effective vaccine. Accelerating the development of a malaria vaccine will be of substantial benefit. The controlled human malaria infection model in semi-immune adults helps overcome a critical block in vaccine development, i.e. comprehensive prioritization of antigens associated with blood-stage immunity for vaccine development. This will be the largest CHMI study in malaria-exposed individuals and will provide data relating to how naturally acquired immunity to malaria depends on specific responses and that overall intensity and/or breadth of antibody responses are associated with immunity. The outcome will determine antigen selection for the next generation of blood-stage malaria vaccines. In our experience of conducting these studies, we found it beneficial to embed social science framework studies to understand perceptions of such type of studies in endemic populations. We implemented this in the second cohort of recruitment and was considered in the first CHMI study conducted in Kenya³⁴. We drew on recommendations from Njue *et al.*³⁷ to shape and change some of the practices of conducting the study. Thus, this study will not only contribute new knowledge on potential correlates of protection in the context of naturally immunity, but the sample set generated will also allow for additional sub-studies to be performed to further unravel our understanding of immunity to malaria.

Furthermore, this will also contribute to our understanding on how to undertake these types of studies through embedded social science sub-studies. This study also aims to build a stronger and more grounded understanding of the ethics of research using the CHMI model to inform future research in this context, and potentially in other similar settings.

Data availability

The extended data have been uploaded to Harvard Dataverse under the title: “Replication Data for: Controlled Human Malaria Infection in Semi-Immune Kenyan Adults (CHMI-SIKA): a study protocol to investigate *in vivo* *Plasmodium falciparum* malaria parasite growth in the context of pre-existing immunity”. DOI: <https://doi.org/10.7910/DVN/XOXLJQ>³⁸.

The files available are listed below:

- [Participant Information sheet and consent for social science sub-study – form 1 study participants](#)
- [Participant Information sheet and consent for social science sub-study – form 2 clinical/trial staff and field workers](#)
- [Participant Information sheet and consent for social science sub-study – form 3 community representatives](#)
- [Social Science sub-study entry questionnaire](#)
- [Social Science sub-study post-challenge questionnaire](#)
- [Case Reporting Form – screening](#)
- [Case Report Form - study](#)
- [Data Safety and Monitoring Committee Charter](#)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license \(CC-BY 4.0\)](#).

Reporting guidelines

SPIRIT Checklist. DOI: <https://doi.org/10.7910/DVN/XOXLJQ>³⁸.

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Salim Abdulla

Ifakara Health Institute, Dar es Salaam, Tanzania

The investigators present a well-designed study using CHMI model to assess the impact of pre-existing antibodies on the blood stage replication of malaria parasites and hence use this indicator to prioritize which antigens to focus on in the malaria vaccine development effort. The investigators point out that so far the blood stage antigens in clinical development have shown modest efficacy and similarly with the sub-unit pre-erythrocytic vaccines. Better results have been seen with the use of whole sporozoite vaccine, generally thought to be due to the broader antigen profile presented as an immunogen. Hence, the idea of identifying other potent sub-unit antigens that can later be used in combination. The investigators mention that they will focus on the already known and well characterized antigens for this work.

Comments and suggestions:

1. The use of the standard CHMI procedures as well as the social science component will further develop this approach for testing new candidate malaria diagnostics, drugs, vaccines and extend the application of the CHMI model into the exploration of the correlates of malaria immunity that is still not very well known.
2. It will be good for the investigators to provide a list of the specific antigens of interest in this study and describe a bit better how this approach of using CHMI platform to select for those antibodies that have been associated with reduced replication will be identified, different from previous work in which antibodies were similarly screened and episodes of parasitaemia monitored. Is the value of the CHMI only about removing the heterogeneity of malaria exposure in the assessment?
3. It will be good for the investigators to comment on the choice of using CHMI with Sporozoite Challenge inoculation approach while the interest is in blood stage antigens and a blood stage Challenge model exist that would have given a more uniform blood stage parasitaemia. I assume that one of the reasons apart from ease of procedures, is the breadth of assessments that can be done using the Sporozoite Challenge approach. Example, pre-patent periods can be assessed as an indicator of liver stage activity. The investigators will take blood samples also during the liver stage replication of the parasites and hence using a battery of immunological assays already proposed to be done, be able to assess both pre-existing as well as newly expressed antibodies for both liver stage as well as blood stage during the follow up period. How this information will be used to identify priority antigens is not very clear in the manuscript. Furthermore, observed high

antibody titres does not always translate into effect in reducing replication so how are the other factors going to be teased out.

4. Is any work going to be done to identify novel antigens during this process?

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Malaria Epidemiology and clinical trials

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 30 Oct 2019

Melissa Kapulu, University Oxford, Oxford, UK

1. The use of the standard CHMI procedures as well as the social science component will further develop this approach for testing new candidate malaria diagnostics, drugs, vaccines and extend the application of the CHMI model into the exploration of the correlates of malaria immunity that is still not very well known.

We thank the reviewer for their comment.

1. It will be good for the investigators to provide a list of the specific antigens of interest in this study and describe a bit better how this approach of using CHMI platform to select for those antibodies that have been associated with reduced replication will be identified, different from previous work in which antibodies were similarly screened and episodes of parasitaemia monitored. Is the value of the CHMI only about removing the heterogeneity of malaria exposure in the assessment?

We have added the reference to the recently published KILCHIP paper Kamuyu et al 2018 describing the antigens. We anticipate that using the CHMI model in the context of pre-existing immunity will help identify potential antigens that could be prioritised for vaccine development. Heterogeneity of exposure is one of the factors that confounds analyses of association between immune responses and outcomes in the field. The CHMI model provides a “clean” system where individuals are equally exposed to a known quantity and genotype of parasites. The known timing of exposure also allows us to calculate the liver to blood inoculum in relation to parasite growth which would not be possible in cohort studies in the field.

1. It will be good for the investigators to comment on the choice of using CHMI with Sporozoite Challenge inoculation approach while the interest is in blood stage antigens and a blood stage Challenge model exist that would have given a more uniform blood stage parasitaemia. I assume that one of the reasons apart from ease of procedures, is the breath

of assessments that can be done using the Sporozoite Challenge approach. Example, pre-patent periods can be assessed as an indicator of liver stage activity. The investigators will take blood samples also during the liver stage replication of the parasites and hence using a battery of immunological assays already proposed to be done, be able to assess both pre-existing as well as newly expressed antibodies for both liver stage as well as blood stage during the follow up period. How this information will be used to identify priority antigens is not very clear in the manuscript. Furthermore, observed high antibody titres does not always translate into effect in reducing replication so how are the other factors going to be teased out.

We refer to our answer to reviewer number 1 comment 3 regarding the use of sporozoites. We agree with the reviewer that high antibody titres do not necessarily translate to a reduction in symptoms or effects on parasite growth. We will examine a wide range of antibody responses, in order to identify the limitations of seeking to explain outcomes with a single response.

1. Is any work going to be done to identify novel antigens during this process?

The antigens described on the microarray (Kamuyu et al 2018) include both previously characterized and uncharacterised and novel antigens. The use of this chip with these antigens is to examine their potential as vaccine candidates at the blood stage with complementary work on functional assays. There are plans to further use the sample set generated to identify “novel” antigens involved in the pre-erythrocytic and transmission stages of the parasite.

Competing Interests: No competing interests

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Alister G. Craig

Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool, UK

This study protocol describes an approach to use a human malaria challenge model to identify lead candidates for blood-stage vaccines, based on screening semi-immune individuals for their ability to reduce parasite growth rates. Many attempts have been made to identify this information from longitudinal studies of patient cohorts but, as the authors point out, these studies have a number of issues, particularly the specificity of the readouts against a wide variation in exposure to infection. The authors seek to address this by using a group of volunteers who will be characterized for their immune status against a large panel of malaria antigens, and subsequently entered into a controlled sporozoite inoculation model of malaria infection to measure parasite growth rates during the erythrocytic cycles allowed in this model.

Controlled human malaria infection (CHMI) is very well-established and provides an excellent platform to conduct experimental medicine research in a safe and acceptable format. The authors follow a careful protocol for the CHMI phase and have access to experts in the field to guide them. In terms of the CHMI aspect of this study protocol, I only have two comments:

1. From the protocol it seems that all volunteers will be treated in Kilifi, but recruited both from Kilifi and Kisumu, the latter being some 870km away. This issue, in terms of how volunteers will be managed, is not discussed in the protocol.
2. The authors say that should intensive care facilities be needed, which is highly unlikely, “a referral will be made to an appropriately equipped facility”. How far away from the testing centre is this facility?

My other comments relate to the study design with respect to the immune correlates:

1. An asexual parasite stage inoculation based CHMI model is also available. Why are the authors using sporozoites, where there could be variability in the merozoite outputs from the liver stages, particularly in semi-immune individuals? In some ways this can be controlled for by having the first cycle parasitaemia as a baseline so that growth rates can still be measured, but it may introduce variation in the erythrocytic ‘inoculum’ size.
2. The definition of ‘semi-immunity’ is not well described and it would be helpful to have some preliminary data on the levels of antibodies seen in the trial populations. This would provide some structure to the decision on who will be recruited based on antibody levels. At the moment the only comment on this is that a “range of volunteers with low and high antibodies to each antigen tested” will be recruited.
3. Along similar lines, from other vaccine trials and harmonized growth inhibitory assays (GIA), we now know that antigens vary in the level of antibodies needed to control parasite growth. For example, the EC50s of MSP1, AMA1 and RH5 are 500ug/ml, 100ug/ml and 10ug/ml respectively. Given this wide variation, how will the parameters for antibody/ antigen selection be derived? Would there be a minimum cut-off below which a volunteer would be excluded from the trial as they would not be expected to show any control of parasite growth? This is a complicated question as we think that antigens may not act independently in controlling parasitaemia, so lower level combinatorial effects cannot be excluded, but it may be prudent to remove very low responders to limit the amount of uninformative data and the risk to a volunteer from whom the expectation might be that the information derived from CHMI would not be useful in designing future vaccines (inasmuch as this can be predicted).
4. An appendix describing the antigens to be used for screening volunteers would be useful. Will different variants of proteins be incorporated or just those for NF54 (the challenge strain)?
5. The range of analytical tools to be used to measure various aspects of host immunity is impressive, including phenotypic and transcriptional approaches. A table to summarize these assays and provide references to their implementation elsewhere would be helpful in determining their likely efficacy. Many of the assays are relatively common, but others are less so, at least to me (e.g. impact on dendritic cells; sporozoite inhibition).
6. The analytical part will generate a huge body of data, which provides two challenges:
 1. How will the database cope with the different forms of information?
 2. Does the power calculation take into account the multiplicity of parameters?

Several of the authors have been involved in complex cohort studies on malaria immunity in Kilifi – have these generated an analytical framework that could be applied to this study?

1. Why are parasite transcriptomes being performed? How will these data contribute to identifying immune signatures of protection? If this is just to differentiate community acquired infection from the CHMI strain NF54, this can be done in a much less complicated way.

Finally, it is good to see the social science research nested within the project and these outcomes will provide useful information. In terms of reporting this and other information, the authors say that they will make the data publicly available “as rapidly as possible”. Would they be willing to put a time limit on this?

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: malaria cytoadherence and pathology of cerebral malaria

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 30 Oct 2019

Melissa Kapulu, University Oxford, Oxford, UK

1. From the protocol it seems that all volunteers will be treated in Kilifi, but recruited both from Kilifi and Kisumu, the latter being some 870km away. This issue, in terms of how volunteers will be managed, is not discussed in the protocol.

Transport to Kilifi and back from Kilifi is organized and paid for by the research project. Participants from Kisumu will be screened for eligibility in Kisumu, then travel to Kilifi for participation in challenge, stay for the duration of the study, then return to Kisumu.

1. The authors say that should intensive care facilities be needed, which is highly unlikely, “a referral will be made to an appropriately equipped facility”. How far away from the testing centre is this facility?

The facilities are in Mombasa are 65km away. We agree intensive care is highly unlikely to be required, although a very low threshold would be used for organizing early transfer to a hospital with intensive care facilities.

1. An asexual parasite stage inoculation based CHMI model is also available. Why are the authors using sporozoites, where there could be variability in the merozoite outputs from the liver stages, particularly in semi-immune individuals? In some ways this can be controlled for by having the first cycle parasitaemia as a baseline so that growth rates can still be measured, but it may introduce variation in the erythrocytic ‘inoculum’ size.

Cryopreserved sporozoites are GMP products that are standardized, produced at scale and acceptable to our community. There is a scientific advantage in being able to assess pre-erythrocytic immunity as well as blood-stage immunity. We agree with the reviewer that blood-stage challenge may reduce the variability in inoculum likely to be observed with sporozoites. We have not yet considered the acceptability of a blood product to our local community and would need to conduct further engagement to establish this.

1. The definition of ‘semi-immunity’ is not well described and it would be helpful to have some preliminary data on the levels of antibodies seen in the trial populations. This would provide some structure to the decision on who will be recruited based on antibody levels. At the

moment the only comment on this is that a “range of volunteers with low and high antibodies to each antigen tested” will be recruited.

We do not believe there is an adequate definition of semi-immunity based on laboratory assays. As a pragmatic option we will use geographical location of residence and responses to schizont extract to stratify according to tertiles. The statement in reference to this under study design has been modified and clarified to read testing of antibodies to schizont extract, as follows: “We will screen all volunteers for varying degrees of immunity, ensuring a range of volunteers with low and high antibody responses to schizont extract.”

1. Along similar lines, from other vaccine trials and harmonized growth inhibitory assays (GIA), we now know that antigens vary in the level of antibodies needed to control parasite growth. For example, the EC50s of MSP1, AMA1 and RH5 are 500ug/ml, 100ug/ml and 10ug/ml respectively. Given this wide variation, how will the parameters for antibody/ antigen selection be derived? Would there be a minimum cut-off below which a volunteer would be excluded from the trial as they would not be expected to show any control of parasite growth? This is a complicated question as we think that antigens may not act independently in controlling parasitaemia, so lower level combinatorial effects cannot be excluded, but it may be prudent to remove very low responders to limit the amount of uninformative data and the risk to a volunteer from whom the expectation might be that the information derived from CHMI would not be useful in designing future vaccines (in as much as this can be predicted).

We agree with the reviewer that these are important and complex questions. Our approach will be to ensure that the volunteers enrolled have a range of responses to schizont extract as a proxy for other responses, and to then examine the range of measured responses to recombinant antigen and functional assays for both linear trends and thresholds. The functional work proposed will not be used to select individuals for enrolment, but rather to correlate infection outcome with functional and antibody responses. Since adults in our setting have a very low rate of febrile malaria episodes following universal exposure to malaria in childhood, we do not expect uninformative very low responders to be present in our population. We agree that combinatorial effects are important to study.

The following paragraph has been added to the assessment of immunity to malaria section which highlights especially the issue of combinations with Osier et al 2014 as an example of combinations of antigen analysis: “In further exploratory analysis we will also undertake principal component analyses to determine if there are typical “signatures” of protective responses, and analyses for combinations of protective antigens as previously described by Osier et al 2014. The statistical significance of protective antigen combinations will be tested using interaction terms and summary metrics as in previous studies [Osier F et al 2014, Bejon P et al 2011].”

1. An appendix describing the antigens to be used for screening volunteers would be useful. Will different variants of proteins be incorporated or just those for NF54 (the challenge strain)?

We have added the reference to the recently published KILCHIP paper Kamuyu et al 2018 describing the antigens. We anticipate many further analyses internally and with external parties which are yet to be defined.

1. The range of analytical tools to be used to measure various aspects of host immunity is impressive, including phenotypic and transcriptional approaches. A table to summarize these assays and provide references to their implementation elsewhere would be helpful in determining their likely efficacy. Many of the assays are relatively common, but others are less so, at least to me (e.g. impact on dendritic cells; sporozoite inhibition).

We now include a table (Table 7) to summarise the potential assays to be utilised and their respective references. In addition, the sample set generated will be made available to external

parties for further study.

1. How will the database cope with the different forms of information?

The size of the database will be comparable to previous multidimensional immunological work (ref <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4856877/> and <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3126844/>) and our standard in-house data handling platforms (such as OpenClinica and LabKey) will be sufficient to manage the various datasets.

1. Does the power calculation take into account the multiplicity of parameters?

The power calculation considers multiple variables, including a Bonferroni correction for 100 comparisons. We calculate $n=200$ for 80% power to find a single variable with a correlation coefficient of 0.3.

1. Several of the authors have been involved in complex cohort studies on malaria immunity in Kilifi – have these generated an analytical framework that could be applied to this study?

There will be a deliberate analytical framework generated and applied to the data generated. This will be largely guided by the outcomes that will be observed from parasite qPCR results. For instance, for parasite growth rates and liver-to-blood inoculums (i.e. the number of parasites exiting the liver and infected red blood cells), these will be calculated by fitting established models to quantitative PCR data. The growth rate will be regressed against the individual immunological measures such as intensity and/or breadth of antibody responses to parasite antigens. Given the co-linearity of responses, a multivariable model is likely to be more informative in testing our hypotheses that: naturally acquired immunity to malaria depends on responses to specific identifiable antigens; and the overall intensity and/or breadth of antibody responses to parasite antigens are associated with immunity. Rather than simply enter all variables in a single multivariable model, we will consider entering variables in clusters and take forward the independent predictors to a final multivariable model. These clusters will be pre-defined and be based on: (a) biological links (for instance grouping all red cell surface antigens separately from merozoite antigens); and (b) on co-linearity (i.e. on clustering groups of antigens with greatest cross-correlations). We will score breadth as the number of antigen responses in the top quartile and mean intensity as the mean of normalized responses for each antigen. These scores will be determined separately for merozoite antigens and for red-cell-surface antigens. We will also incorporate functional assays, anti-circumsporozoite antibody levels, responses to schizont extract, and scores for mean intensity and breadth of response across antigens which will be added to the final model to test the hypotheses mentioned above. To test the hypothesis that there are specific thresholds above which immunological responses control parasite growth, we will examine this final model for non-linearity using multiple fractional polynomials and Hill functions to determine if there are discrete thresholds predicting efficacy according to previously used methods.

We have added text to the section on statistical and analytical plans.

1. Why are parasite transcriptomes being performed? How will these data contribute to identifying immune signatures of protection? If this is just to differentiate community acquired infection from the CHMI strain NF54, this can be done in a much less complicated way.

Parasite transcriptomes mainly for looking at var gene changes in relation to immunity as previously described by Abdi et al 2017 (BMC Infect Dis), and also examining commitment to transmission stages as secondary aims.

1. Finally, it is good to see the social science research nested within the project and these outcomes will provide useful information. In terms of reporting this and other information, the authors say that they will make the data publicly available “as rapidly as possible”. Would they be willing to put a time limit on this?

This will be done in parallel with the primary publications arising from the work. We have already begun to make the output of this work readily available as rapidly as possible (refer to Njue et al 2018 Wellcome Open).

Competing Interests: No competing interests