CONFIDENTIAL

CLINICAL TRIAL PROTOCOL

Controlled Human Malaria Infection models for evaluation of *Plasmodium falciparum* transmission-blocking interventions in healthy Malian adults

Version ~~1~~2.0 ~~, 24 May 2021~~17 February 2022

PROTOCOL TITLE: 'Controlled Human Malaria Infection models for evaluation of *Plasmodium falciparum* transmission-blocking interventions in healthy Malian adults'

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Investigator Agreement

"I have read this protocol and agree to abide by all provisions set forth therein.
I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

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Version History

Version	Date	Author(s)	Summary of changes
1.0	~~dd Month yyyy~~24 May 2021		First version
2.0	17 February 2021		- Second version

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

3D7	***P. falciparum* 3D7 strain**
An.	***Anopheles***
AE	**Adverse Event**
AL	**artemether–lumefantrine**
ALT	**Alanine Aminotransferase**
ANOVA	**analysis of variance**
BMI	**Body mass index**
BP	**Blood pressure**
CHMI	**Controlled Human Malaria Infection**
CHMI-Trans	**Controlled Human Malaria Infection Transmission Model**
CRF	**Case Report Form**
D(S)F(A)	**Direct (Skin) Feed(ing Assay)**
DHA	**Dihydroartemisinin**
DHA-PQP	**Dihydroartemisinin - piperaquine phosphate**
DMFA	**Direct Membrane Feeding Assay**
DSMB	**Data and Safety and Monitoring Board**
EC	**Ethics Committee**
ECG	**Electrocardiogram**
EDCTP	**European and Developing Countries Clinical Trials Partnership**
EDTA	**Ethylenediaminetetraacetic acid**
EOS	**End of Study**
eCRF	**electronic Case Report Form**
ELISA	**Enzyme-Linked Immuno Sorbent Assay**
FMPOS	**Faculty of Medicine, Pharmacy, and Dentistry**
G6PD	**Glucose-6-phophatedehydrogenase deficiency**
GCP	**Good Clinical Practice**
HBV	**Hepatitis B Virus**
HCV	**Hepatitis C Virus**
HIV	**Human Immunodeficiency Virus**
IBSM	**Induced Blood Stage Malaria**
IRB	**Institutional Review Board**
ITN	**insecticide-impregnated bednets**
IV	**Intravenous**
LMIC	**Low and middle income country**
LMIV	**Laboratory of Malaria Immunology and Vaccinology**
LSM	**Local Safety Monitor**
MRTC	**Malaria Research and Training Center**
NaCl	**Sodium Chloride**
NF54	**Nijmegen *falciparum* strain 54**
NIAID	**National Institute of Allergy and Infectious Diseases**
NIH	**National Institutes of Health**
P.	***Plasmodium***
p/µl	**parasites per microliter**
PBMC	**Peripheral blood mononuclear cell**
PCR	**Polymerase Chain Reaction**
Pf	***Plasmodium falciparum***
PhHV	**Phocine Herpes Virus**
PIP	**Piperaquine**
qPCR	**Real-time Quantitative Polymerase Chain Reaction**

qRT-PCR	**Quantitative Reverse Transcriptase Polymerase Chain Reaction**
QIMR-B	**QIMR Berghofer Medical Reseach Institute**
Radboudumc	**Radboud university medical center**
SAE	**Serious Adverse Event**
SMC	**Safety Monitoring Committee**
SMFA	**Standard Membrane Feeding Assay**
SOP	**Standard Operating Procedure**
SPC	**Summary of Product Characteristics**
SPZ	**Sporozoites**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, and is referred to here as a funding partner.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
T1	**Treatment 1**
T2	**Treatment 2**
T3	**Treatment 3**
TBV(s)	**Transmission Blocking Vaccine(s)**
USTTB	**University of Sciences, Techniques and Technologies of Bamako**
WHO	**World Health Organization**

SUMMARY

Rationale:
Malaria remains one the leading infectious causes of morbidity and mortality worldwide. Despite progress in reducing the burden of malarial disease during the first decade of the 21st century, in 2019 there were still 229 million cases and 405,000 deaths, mainly *Plasmodium falciparum* (Pf) infections in children under five years of age in sub-Saharan Africa [1]. In addition to the direct clinical burden, malaria represents a significant economic burden to affected countries, most of which are low and middle income countries (LMIC). Further urgency is instilled by the waning effectiveness of currently deployed control measures, as witnessed by the emergence of parasite isolates resistant to anti-malarial drugs and mosquito isolates resistant to insecticides. The former are under strong positive selection, leading to their ever-increasing contribution to transmission [2]. Transmission-blocking interventions aim to prevent vector-borne onward transmission of malaria parasites from an infected individual to others, thus reducing the incidence of new infections and overall morbidity and mortality. Malaria transmission blocking vaccines (TBVs) aim to induce immune responses that interrupt the development of sexual-stage parasites in the Anopheline mosquito vector [3], thus preventing onward transmission. In addition to reducing the burden of malaria, TBVs may play a central role in containing the spread of drug-resistant parasites and form a valuable tool on the final path towards malaria elimination [2, 4].

As an essential step in the clinical development of TBVs and other transmission-blocking interventions (TBIs), efficacy is ultimately assessed as the ability to inhibit transmission of naturally-acquired infections in endemic populations. Due to heterogeneity in exposure, in combination with other confounders such as naturally-acquired immune responses against pre-erythrocytic and asexual blood-stage parasites, it remains unpredictable which participants will develop gametocytemia and when, if at all. Large, lengthy trials are therefore required to evaluate transmission-blocking interventions, making it difficult to test multiple TBI candidates.
Controlled Human Malaria Infection (CHMI) models are an established methodology for evaluating the efficacy of pre-erythrocytic and asexual blood-stage malaria vaccine candidates and drugs. Controlled *P. falciparum* infection can be induced either by sporozoites (through the bites of Pf-infected mosquitoes or direct venous inoculation of purified, aseptic, cryopreserved, infectious *P. falciparum* sporozoites [PfSPZ Challenge]), or asexual blood-stage parasites (through intravenous inoculation of cryopreserved Pf-infected erythrocytes, [IBSM Challenge]). Recently, we developed a controlled human malaria infection transmission model or "challenge model", in which *Plasmodium falciparum* (*Pf*) gametocyte carriage is induced in malaria-naive participants and the gametocytes are transmitted to *Anopheles* mosquitoes [5, 6]. Such a model forms a powerful tool to evaluate the transmission-reducing and -blocking capacity of vaccines, biologicals (monoclonal antibodies) and drugs, eliminating the two main confounders described above and accelerating clinical development by allowing rapid down-selection of the most promising candidates in small pilot studies.
As a bridge to ultimate field trials, it is necessary to transfer the CHMI-transmission model to endemic settings for the evaluation of transmission-blocking interventions in the target population – lifelong-exposed individuals. The primary aim of this project is to develop and optimise a CHMI-transmission model in Mali ("*CHMI-trans Mali*"), an area of intense seasonal malaria. Once developed we can utilize this model to accelerate the evaluation of transmission-blocking interventions, down selecting the most promising interventions for evaluation against naturally-acquired infections.

Objectives:
Primary objectives:
1) Primary safety objective: To evaluate the safety and tolerability of the CHMI-transmission model in healthy Malian adult participants inoculated intravenously with either sporozoites (PfSPZ Challenge) or asexual blood-stages of *Plasmodium falciparum* (IBSM Challenge).

2) Primary efficacy objective: To determine the prevalence and kinetics of gametocytemia in healthy Malian adult participants inoculated intravenously with either sporozoites (PfSPZ Challenge) or asexual blood-stages of *Plasmodium falciparum* (IBSM Challenge).

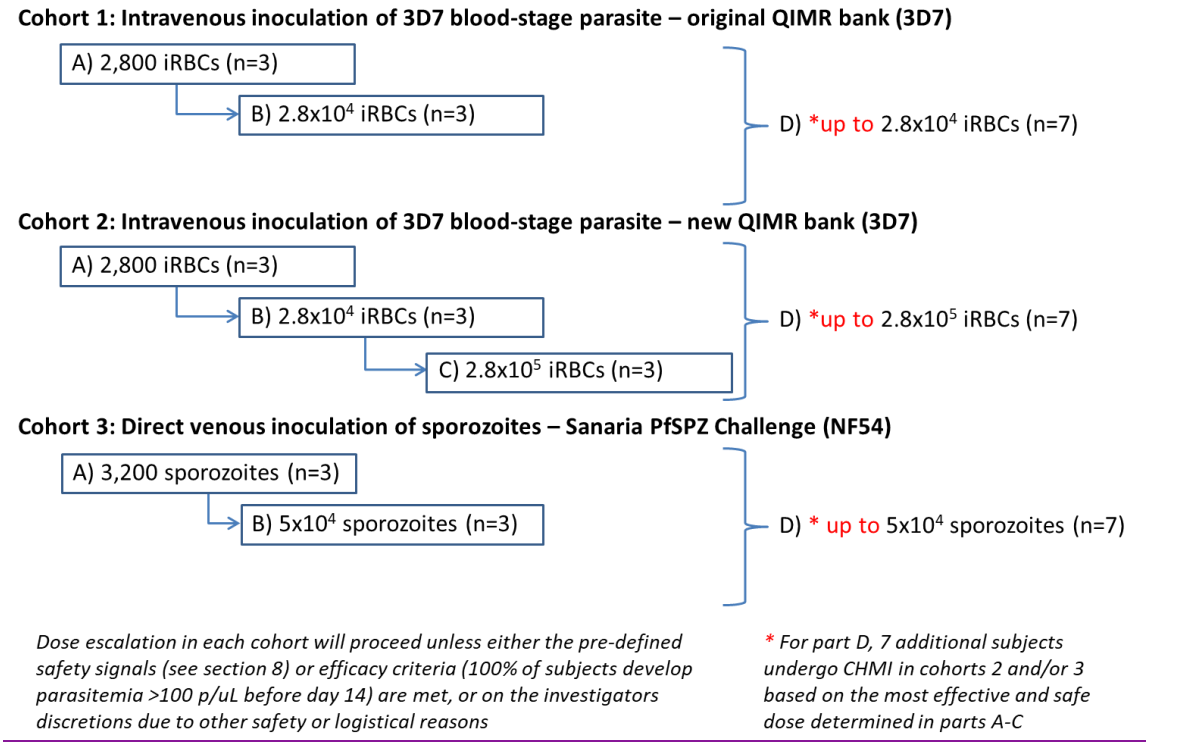
Secondary objectives:
1) To determine the prevalence and kinetics of parasitemia in healthy Malian adult participants inoculated intravenously with either sporozoites (PfSPZ Challenge) or asexual blood-stages of *Plasmodium falciparum* (IBSM Challenge).
2) To assess the infectiousness of participants to *Anopheles* mosquitoes through Direct Skin Feeding Assay (DFA) and/or Direct Membrane Feeding Assay (DMFA).

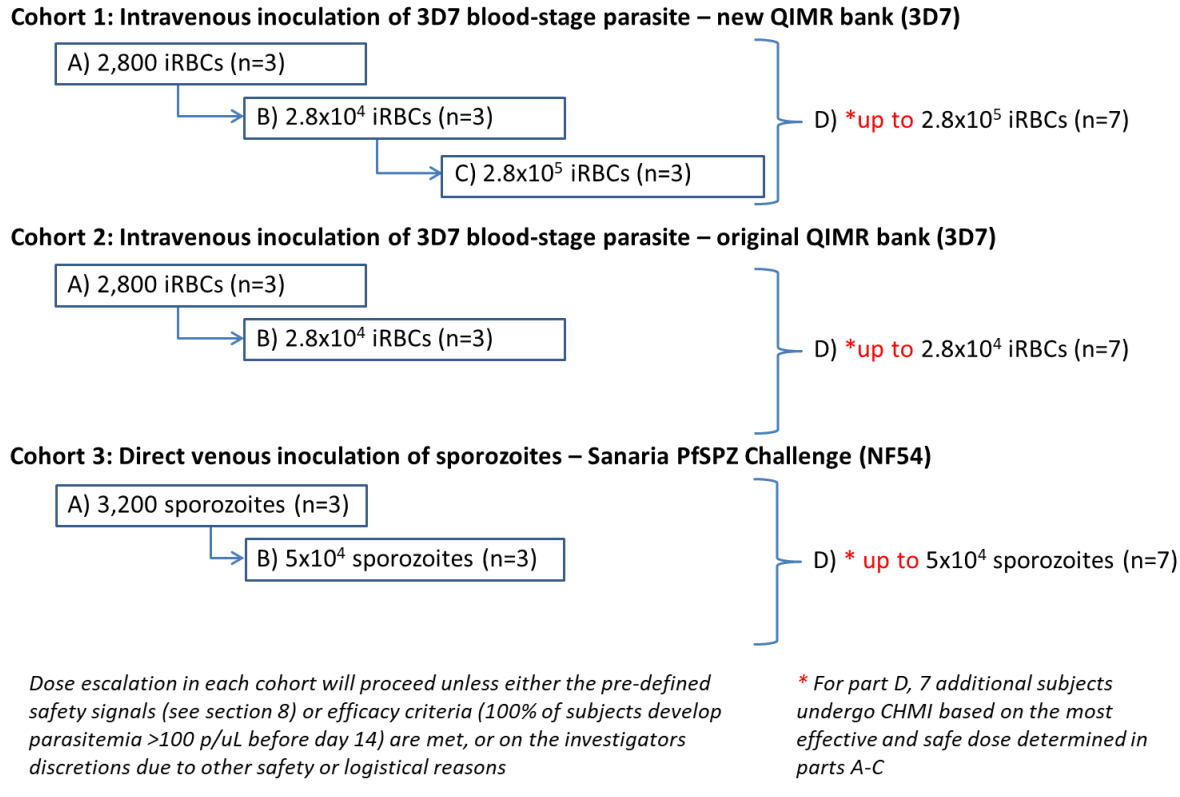
Exploratory Objectives:
1) To determine the optimal parameters for the CHMI-transmission model in this population (inoculum type and dose, and timing of mosquito feeds), based on parasite/gametocyte kinetics and provisional DFA/DMFA results
2) To determine the required sample size for a CHMI-transmission study to evaluate efficacy of transmission-blocking interventions.
3) To assess the dynamics of gametocyte commitment, maturation and gametocyte sex ratio.
4) To assess transmission reducing activity (TRA) of participants' sera at baseline in Standard Membrane Feeding Assays (SMFAs) and assess correlations with DFA/DMFA during CHMI-transmission model
5) To assess correlations between baseline markers of malaria-exposure and asexual and sexual stage immunity with parasite and gametocyte kinetics during the CHMI-transmission model
6) To analyse immune responses in participants inoculated intravenously with either sporozoites (PfSPZ Challenge) or asexual blood-stages of *Plasmodium falciparum*

Study design:
Single center, open label, sporozoite and blood-stage challenge study.

Study population:
A maximum of 42 healthy male participants, aged 18 to 50 years, will participate in the study.

Intervention:
A maximum of 42 participants will be recruited following screening. ~~and serological profiling for low levels of cumulative malaria exposure [7].~~ Participants will be assigned to one of the following study (sub-) cohorts.





Participants in **Cohort 1 and 2** will undergo controlled human malaria infection (CHMI) through a standard blood-stage challenge with *Pf*-infected erythrocytes by intravenous injection. Participants in **Cohort 3** will undergo a CHMI through a standard sporozoite challenge with PfSPZ Challenge (NF54) by direct venous inoculation. All cohorts include the potential for dose escalation, and in principle the dose will increase ~10/20-fold between parts (A, B and where applicable C). The starting inoculum dose will be that which has proven to be safe in previous CHMI studies. For the QIMR blood-stage inoculums this is ~2,800 viable parasites in malaria-naïve participants [5, 6] [8]. For PfSPZ Challenge

this is 3,200 sporozoites both in malaria-naïve participants (clinical trials.gov: NCT01624961 [9]) and pre-exposed adults in Mali [10]. Each cohort may also include a consolidatory part (D), using the most effective and safe dose determined in parts A-C. Parts A, B, (C) and D for each cohort will be conducted sequentially, a minimum of 14 days apart. Dose escalation within each cohort will proceed if the efficacy criterion (≥100 p/µL in all subjects before day 14) is not met in the preceding part of that cohort, and in the absence of protocol-defined safety signals up to day 14 (any subject with a grade 3 AE probably or definitely related to CHMI; and/or >2 subjects with a grade 2 AE probably or definitely related to CHMI; and/or any subject with an initial parasitemia ≥100p/µL). If any such safety signals are observed, a~~n~~ data and safety monitoring ~~committee~~ board (~~SMC~~DSMB) meeting will be convened to determine if it is safe to continue with dose escalation in that cohort. If an efficacious dose is identified for a Cohort ~~2 or 3~~ before completing the full dose escalation schedule, the Principal Investigator may elect to challenge the remaining participants in that cohort with that dose, or proceed to part D. Based on data from Parts A and/or B, an ~~SMC~~ DSMB meeting may also be convened to adjust the dose escalation schedule outside of the planned 10-20 fold dose increases (up to maximally 100-fold dose escalation at each step). The Principal Investigator may moreover decide to not conduct further dose-escalation and/or consolidation within any individual cohort due to logistical constraints. Treatment with piperaquine will be used when necessary to clear pathogenic asexual parasites whilst leaving gametocytes unaffected. Piperaquine treatment is critical to preserve viable gametocytes and allow assessment of transmission endpoints. Treatment with low dose piperaquine (T1, 480 mg) will be initiated when either; (a) parasitemia reaches 1000 p/µL (in line with other CHMI studies in Africa) [11, 12], or (b) if a participant develops signs or symptoms of malaria accompanied by a positive thick-film blood smear (TBS). The initial clearance of parasitemia will be carefully monitored using daily blood samples. If recrudescent asexual parasitemia develops ≥1000 p/µL, a second, higher dose of piperaquine (T2, 960mg) will be administered. These treatment regimens cure asexual parasitemia while leaving immature and mature gametocytes unaffected [13]. Clinical decisions will be based on duplicate microscopy readings. Venous blood samples will be collected for Direct Membrane Feeding Assays (DMFAs) and/or to Direct Skin Feeding Assays (DFAs) per MRTC SOPs on up to 3 occasions, the exact time-point being based on the density of gametocytemia as measured by gametocyte qRT-PCR and/or microscopy. These assays will provide evidence of the infectivity of participants to mosquitoes at these time-points. At the end of study (day 49), or if a second recrudescence of asexual parasitemia develops >1000 p/µL, or if there is a safety concern or adverse event that in the opinion of the safety monitor or investigator requires immediate curative antimalarial treatment, participants will receive end of study treatment (T3, artemether/ lumefantrine and low-dose primaquine) to ensure they are parasite and gametocyte free.

Participants will be confirmed parasite free 1 to 3 days before challenge by qPCR and assessed again on the day of challenge. The challenge study will take place in a setting (season, location and accommodation) designed to minimise exposure to environmental mosquitoes and participants will be required to remain in the study area for the duration of CHMI follow-up.

In case a participant remains parasite negative by TBS for ~~19~~ 21 days after ~~blood-stage challenge or 21 days after sporozoite~~ challenge, frequency of follow-up visits will be reduced to ~3x/week. For those remaining negative by PCR until 2~~8~~6 days after ~~blood-stage challenge or 28 days after sporozoite~~ challenge, end of the study treatment will be given and end of study will apply for that participant.

Main study parameters/endpoints:

Primary endpoints:

- Frequency and severity of adverse events in the CHMI-transmission study participants in each cohort

- Prevalence and density of gametocytes as determined by qPCR and/or thick-film blood smear (TBS) microscopy by cohort

Secondary endpoints:
- Prevalence and density of parasitemia as determined by qRT-PCR and/or TBS microscopy by cohort
- Proportion of infected *Anopheles* mosquitoes following DFA/DMFA in each cohort
- Intensity of oocyst infection in mosquitoes following DFA/DMFA in each cohort
- The sample size required to evaluate the efficacy of transmission blocking interventions in this population determined through mathematical modeling of the parasite/gametocyte kinetics and DFA/DMFA results

Exploratory endpoints:
- Gametocyte commitment and maturation rates and gametocyte sex ratio determined by molecular markers of sexual stage development
- Gametocyte fitness assessed by ex vivo gamete formation and fertilization assays
- Transmission reducing activity of participants' baseline sera measured by reduction in prevalence of infected *Anopheles* mosquitoes and intensity of oocyst infection in SMFAs.
- Baseline serum antibody levels against a panel of malaria antigens that may include (but is not limited to) AMA-1, MSP-1.19 and GLURP.R2, GEXP18, Rh2.2030, Etramp5.Ag1, Pfs230 and Pfs48/45, and/or malaria schizont extract.
- Induction of humoral and cellular immune responses by the CHMI-transmission model

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:
Benefits: There are no direct benefits for participants. Participants will be advised to seek medical help when suffering from malaria signs or symptoms in the future. Participants will be compensated for the time lost due to study participation

Risks: the risks for individual participants are related to (i) exposure to either infectious *P. falciparum* sporozoites malaria *or P. falciparum* infected erythrocytes and (ii) potential side-effects associated with treatment medications.

The IV injection of small numbers of sporozoites (e.g., 3,200) or *Pf*-infected erythrocytes (up to ~20,000) is not known to cause any side effects other than the risks of minor local bruising. Subjects will be closely monitored during and after administration by clinical staff trained and equipped to respond to anaphylaxis, although this remains extremely unlikely to occur based on previous studies. Dose-escalation will take place only if required to achieve efficacy goals and in the absence of significant safety signals in the respective lower dose group.

Subjects who undergo CHMI may develop symptoms associated with mild, uncomplicated malaria. Like naturally-acquired malaria, CHMI may theoretically progress to a serious, life-threatening illness if not promptly diagnosed and treated. However, this risk is negligible if parasitaemia is diagnosed and treated early. Since the advent of the modern era of CHMI, severe, life-threatening malaria has never occurred in CHMI studies.

Amongst malaria-naïve subjects undergoing CHMI by mosquito bite at Radboudumc, a number of cases of cardiac SAE have occurred of uncertain aetiology. No cases have until now occurred following mosquito bite CHMI at other centres, or following CHMI induced by inoculation of PfSPZ Challenge, but other non-serious cardiac adverse events have occasionally occurred following CHMI induced by *Pf*-infected erythrocytes. Asymptomatic self-limited liver function test abnormalities

(including grade 2, 3 and 4 adverse events) have also been observed following mosquito bite, PfSPZ and IBSM challenge in malaria-naïve subjects. See section 1.4 for further details on risk and mitigation measures.

The risk for the community stems from the potential onward transmission from gametocytemic participants to local mosquitoes. This is mitigated by performing these studies at a time (season) and in a location where local transmission is very low, and ensuring participants have appropriate accommodation, insect repellant, and sleep under new insecticide-treated nets during the study period to reduce any risk of mosquito bites in the community. All participants must demonstrate an understanding of these requirements prior to enrolment.

Burden:
The study involves a malaria infection by inoculation of aseptic, purified, cryopreserved Pf sporozoites (PfSPZ Challenge (Pf NF54 strain)) or inoculation of infected RBCs (Pf 3D7 clone). To evaluate transmission blocking interventions, this study must find a balance between inducing sufficient parasitemia to produce gametocytemia and reducing the risk of symptoms caused by asexual stage parasites. After the challenge there will be a period (49 days) of intense clinical monitoring with frequent visits (up to two times a day) and blood examinations. Subjects will receive treatment with piperaquine (480mg) to clear asexual parasites, but allow ongoing development of gametocytes either when parasitemia reaches 1000 parasites/µL or when symptoms of malaria develop (Appendix 1) in combination with any density of *P. falciparum* parasitemia detected by thick-film blood smear (T1). Initial clearance of parasitemia will be carefully monitored using blood samples taken daily. If recrudescent asexual parasitemia develops ≥1000 p/µL, a 960 mg dose of piperaquine will be administered (T2). If a participant experiences mild symptoms of malaria while asexual parasites are being cleared, anti-inflammatory medications such as ibuprofen or paracetamol may be administered for comfort. Tolerance of mild symptoms under supervision while asexual parasites are being cleared allows for the development of sufficient gametocytemia to study parasite transmission. On day 49, or if a second recrudescence ≥1000 p/µL occurs, or if there is a safety concern or adverse event that in the opinion of the safety monitor or investigator requires immediate curative antimalarial treatment, participants will be treated with end of study treatment (artemether/lumefantrine and low dose primaquine) to assure the clearance of all parasite stages. The exact number of site visits and blood examinations per participant depends on the time to development of parasitemia above 1000 p/µL and potential recrudescence - with a maximum number of 45 planned study visits and a maximum of 500 mL collected blood. Periodic physical examinations and blood examinations will be performed, including up to twice-daily monitoring during parasitemia. In addition, three direct skin feeds (DFA) will be performed as per MRTC SOPs. The duration of participation will be 49 days from day of challenge, following a screening period of up to 120 days.

1. INTRODUCTION AND RATIONALE

1.1. Introduction

Malaria is one of the most devastating infectious diseases worldwide. Despite progress made in reducing the burden of malaria by the up-scaling of protective measures and efficacious treatment [14], in 2019 there were still 229 million cases and 409,000 deaths, with children under five years of age in sub-Saharan Africa most severely affected [1]. In addition to the considerable human suffering, this disease exerts a significant economic burden for the affected countries, which are already struggling with poverty. The urgency of the situation is further highlighted by the waning effectiveness of all currently registered anti-malarials, due to emergence and spread of resistance and the absence of an effective vaccine [2, 15]. The World Health Organization (WHO) has declared malaria control a global development priority and has changed their recommendation from control programs to eradication programs. It is widely accepted that malaria eradication is unlikely to be attainable with the currently available tools [14, 16].

The major challenge for malaria elimination is the highly efficient spread of malaria parasites. Human malaria is caused by protozoa of the genus *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Parasites (sporozoite stage) are injected into the skin by an infected female *Anopheles* mosquito. After penetration of the skin capillaries, sporozoites are transported to the liver, where they develop and multiply in liver cells before emerging into the blood (merozoite stage) and invading red blood cells for further maturation and multiplication. The cyclical proliferation of asexual stages within the human red blood cells is responsible for the occurrence of clinical symptoms. During each replication cycle a small fraction of asexual stage parasites commit to becoming sexual stage parasites (gametocytes). The formation of male and female gametocytes is essential for parasite transmission via the female *Anopheles* mosquito vector. Circulating gametocytes do not cause clinical pathology or symptoms. The circulating gametocytes therefore have no clinical consequences but play an essential role in the onward transmission of malaria. The renewed focus on malaria elimination has increased the priority of research towards development of interventions to block malaria transmission, including transmission blocking vaccines (TBVs). By interrupting transmission of malaria parasites to mosquito vectors, a reduction in the number of secondary infections in the community is expected. TBVs will play an important role in complete arrest of malaria transmission in endemic areas [4, 17]. Similarly, a number of gametocidal and/or sporontocidal drug candidates have been generated in recent years [18]. From a community perspective, deployment of transmission-blocking interventions (TBIs) will be an efficient complementary element in an integrated program of anti-malarial interventions, particularly for malaria elimination.

1.2. Rationale

The first step of malaria parasite transmission from humans to *Anopheles* mosquitoes, is the generation of mature gametocytes in the human peripheral blood. Gametocytes are non-pathogenic malaria life stages and typically comprise less than 5 percent of the total parasite population prior to treatment. Once treatment is initiated, gametocyte production either ceases abruptly (e.g. artemisinins) or is tolerated or potentially even stimulated as part of a terminal investment of malaria parasites under drug pressure (e.g. sulfadoxine-pyrimethamine and piperaquine) [13, 19, 20]. Importantly, malaria transmission is not prevented by currently used antimalarial drugs or the recently proposed malaria vaccine RTS,S. Malaria TBIs can interrupt parasite development in the mosquito and thereby play a central role in malaria elimination efforts and in efforts to contain the spread of drug resistant malaria [4, 21]. However, for downstream selection and clinical development, there is currently an uncertain association between the methods used for preclinical and early clinical evaluation of candidate drugs/vaccines (e.g., the standard membrane feeding assay, or SMFA) and their ultimate field deployment, where the infectivity of naturally exposed hosts forms a key outcome measure. The standard membrane feeding assay (SMFA) can determine the

percentage of the transmission-reducing activity (TRA) of human serum by feeding Anopheles mosquitoes on human blood with cultured gametocytes, mixed with experimental sera. Presently, we are heavily reliant on the SMFA to inform the early clinical efficacy stage-gate; however, this assay has not been accurately calibrated against the most widely used ex vivo assessment of gametocyte infectiousness, the direct membrane feeding assay (DMFA) [22]. Therefore, its predictive value in assessing interventions that block human-to-mosquito transmission remains uncertain [3].

Controlled human malaria infection (CHMI) studies have become a safe [23-25] and widely accepted model for evaluating the efficacy of vaccines [26], anti-malarial drug candidates [27-29], diagnostic assays [30] and assessment of immunologic responses [31, 32]. CHMI can be initiated either by sporozoites via the bites of infected mosquitoes or injection of cryopreserved sporozoites, or by induced blood-stage malaria (IBSM) infection, where *P. falciparum*-infected erythrocytes are administered intravenously. These studies provide a cost-effective and fast way to circumvent the use of large-scale field efficacy studies for down selection of intervention candidates.

Until recently, CHMI models did not allow assessment of gametocytes and infectivity to mosquitoes, in part due to the requirement for both state of the art clinical and mosquito facilities in the same location. In the last 4 years, we have successfully developed a CHMI-transmission model for *P. falciparum* (Figure 1) in malaria naive individuals, specifically to evaluate the capacity of vaccines, biologics (monoclonal antibodies), and drugs to block malaria parasite transmission by assessing infectiousness of *Plasmodium falciparum* (*Pf*) gametocyte carriers to *Anopheles* mosquitoes [5, 6, 33]. We have demonstrated that gametocytemia can be safely and reproducibly induced in participants using both mosquito-bite and blood-stage inoculation, and successful onward transmission to laboratory mosquitoes was achieved using direct skin feeding and direct membrane feeding assays for the majority of participants following blood-stage inoculation [5, 6]. These studies showed that gametocyte densities are strongly associated with the preceding densities of the asexual parasite progenitors. Therefore, the model could be further optimized to maximize gametocyte densities and the area under the curve (AUC) of gametocytemia by increasing the duration and load of the asexual parasite burden (asexual AUC) without compromising the safety of the participants.

Here, we propose to build on our successes and establish a more relevant CHMI-transmission model in the target population in a malaria-endemic area. Adaptation of the CHMI-transmission model to a pre-exposed population requires consideration of the impact of pre-existing immunity on parasite and gametocyte kinetics. Some level of immunity may be beneficial by controlling infection and safely permitting an overall greater biomass of asexual parasitemia, and in turn gametocytemia, thus optimizing the model. However, strong levels of immunity may completely abrogate parasite development in a proportion of participants upon inoculation. We therefore propose 2 approaches to account for pre-existing immunity. Firstly, we will identify study participants with lower levels of anti-blood stage immunity using ~~an established panel of antigens as~~ serological markers of ~~recent and cumulative~~ *P. falciparum* exposure [34] and ~~or anti-schizont ELISAs, and in addition,~~ identify those with low responses to anti-gametocyte antigens ~~(e.g. Pfs48/45, Pfs230)~~ [35]. This process was applied in a recent standard CHMI study in the Gambia, where low exposed individuals successfully developed asexual parasitemia at similar densities to malaria-naïve individuals [7]. Secondly, we will perform a dose escalation of the parasite-infected red blood cells or sporozoite inoculum to determine the minimal dose required to induce reproducible parasitemia safely in adults in Mali.

Establishment of the CHMI-transmission model in malaria-exposed populations would fill a critical gap in the TBI development pipeline and accelerate development of transmission blocking interventions by allowing more rapid and more relevant evaluation of new TBI candidates in the target population. Moreover, the proposed model will facilitate effective bridging of SMFA to the direct membrane feeding assay (DMFA) and direct skin feeding assay (DFA), and SMFA may become a more informative tool for predicting clinical outcomes.

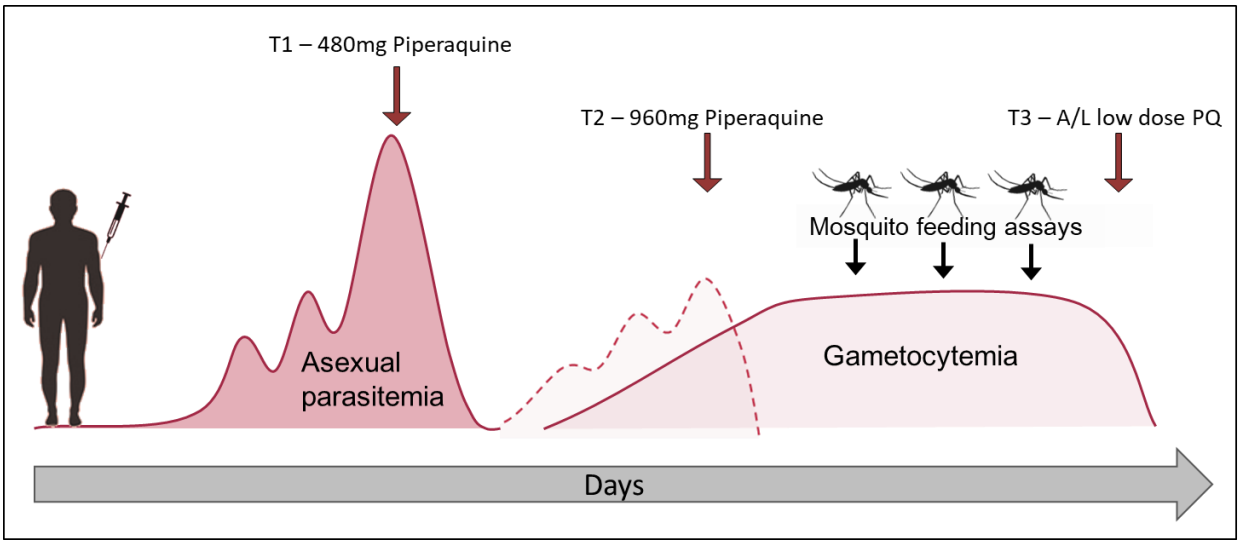


Figure 1. CHMI-transmission model

1.3. Clinical Experience

There is significant clinical experience with infecting humans by the bite of *P. falciparum* sporozoite-infected mosquitoes and blood-stage inoculation of *P. falciparum* infected RBCs. These challenge trials have become highly standardized [36, 37][36, 37][36, 37][36, 37][35, 36][38]. The first human malaria challenge study was performed in 1917, and since 1986, when the modern protocol using laboratory adapted *P. falciparum* strains was first performed by the US army, >3,500 subjects have been challenged by the bites of mosquitoes fed on cultures of *P. falciparum* gametocytes to produce sporozoites [39]. A modern protocol for infecting participants directly with blood-stage parasites using highly-characterised inocula of Pf-infected erythrocytes derived from an infected participant and cryopreserved in a Master Cell Bank was established in 1992 [40]. Over 400 participants have undergone CHMI using this approach [6, 41-44]. More recently this same master cell bank was expanded under GMP conditions to increase the stocks. This bank has been tested in in malaria-naïve participants in a pilot study and shown to have comparable safety and parasite kinetics as the original master cell bank [8]. Finally, the establishment of a method producing GMP-grade aseptic, purified, cryopreserved infectious *P. falciparum* sporozoites (PfSPZ Challenge) that can be shipped in vapour-phase liquid nitrogen and administered by direct venous inoculation has formed a valuable addition to the CHMI toolkit, allowing the conduct of CHMI studies in research centres around the world without established insectaries. This approach has facilitated, in particular, the conduct of CHMI trials in endemic populations in sub-Saharan Africa [7, 11, 12, 45-47]. Infecting humans by the bite of *P. falciparum* sporozoite-infected mosquitoes, blood-stage inoculum and PfSPZ Challenge are now established clinical trial methodologies and are considered a reproducible, predictable and safe method of inducing *P. falciparum* malaria. The results of such studies were summarized in 1997 [23], in 2007 [48] and in 2012 [37, 49].

MRTC has already successfully conducted Good Clinical Practice (GCP)-compliant CHMI trials in Mali using PfSPZ Challenge, involving 44 participants [10]. Further methodological and logistical support will be provided by the Radboud university medical center (Radboudumc). Radboudumc has amassed extensive experience conducting CHMI trials with around 500 subjects having undergone CHMI in studies conducted since 2001, using inoculation by either mosquito-bite, PfSPZ Challenge or Pf-infected erythrocytes (IBSM challenge). Two of these studies were CHMI-transmission trials [5, 33]. At Radboudumc, we have developed a sensitive method of parasite detection by real-time quantitative PCR (18S qPCR) in whole blood that allows detection of parasitemia at an extremely early stage that is able to detect small differences in parasite density [50]. We also further pioneered

assays for molecular detection of low densities of gametocytes [51] and sexing of gametocytes [52] as parameters for the transmission from humans to mosquitoes [53].

1.4. Safety

After CHMI, most malaria-naive participants experience symptoms such as headache, chills or fatigue during 1-3 days, however, in pre-exposed African adults a proportion undergoing CHMI remain asymptomatic even when experiencing parasitemia. During the extensive experience with CHMI, both in naïve and pre-exposed populations, severe or life-threatening malaria has never been reported.

In the course of CHMI studies conducted at Radboudumc, five cardiac events have occurred, all involving participants exposed to Pf sporozoites by infected mosquito bite: four undergoing CHMI and one in the context of an immunizing infection under chloroquine prophylaxis (CPS immunization). No cases of cardiac adverse event have been reported in participants undergoing CHMI by mosquito bite at other centers around the world, or following infection with PfSPZ Challenge, including in all trials involving African adults. Of the cardiac events, the first of these participants (with pre-existing cardiovascular co-morbidity) experienced a myocardial infarction. All other subjects were diagnosed with acute coronary syndrome (ACS), with either a confirmed or differential diagnosis of myocarditis as cause [54, 55]. Although all five cases are strongly temporally associated with study participation, remarkably the occurrence of acute coronary syndrome or myocarditis has not been described in either the hundreds of millions of cases of naturally-acquired uncomplicated malaria that occur globally every year.

These cases share a number of characteristics: i) they took place 1-5 days after start of antimalarial treatment, but with a variety of different anti-malarial drugs (CQ, A/L, A/P), ii) at the time of the event parasitemia was no longer detectable and malaria-related symptoms had generally already subsided, and iii) in some cases, other and known potentially triggering factors (e.g. preceding vaccinations, concomitant infections, cannabis use) were present during or preceding the event.

In addition, following CHMI induced by Pf-infected erythrocytes malaria-naïve volunteers have very occasionally experienced non-serious ventricular extrasystoles attributed to unmasking of a predisposition to benign fever-induced tachyarrhythmia [62].

As a result of these cardiac SAEs, safety procedures for CHMI have been strongly intensified. In the current trial, we will adhere to risk-reduction measures, including:

1. Individuals are excluded from participation if they have an elevated risk for cardiovascular disease, including first or second degree relatives who had cardiovascular events (including ischemic events and myocarditis) under the age of 50
2. Electrocardiography of participants at screening and during the study

Asymptomatic liver function test abnormalities involving self-limited transaminase elevations have been reported in malaria-naïve CHMI participants following PfSPZ, mosquito-bite, and IBSM challenge. While the majority of these adverse events are minor, and similar to those experienced following natural malaria infection, grade 4 elevations in alanine transferase (ALT) have been observed in up to 5% of participants following IBSM challenge [56, 57]. These transient biochemical adverse events typically occur in the 3 to 10 days following administration of antimalarial treatment, are not associated with elevated bilirubin, and may be associated with high asexual parasitemia and cumulative paracetamol (acetaminophen) exposure. Compared to malaria naïve individuals, liver function test abnormalities following natural malaria infection are less common in malaria-experienced individuals [58, 59] and are less common following CHMI in malaria endemic areas (R. Sauerwein, personal correspondence).

Moreover, to reduce the risk of LFT elevations during CHMI we will adhere to risk-reduction measures, including:

1. Exclusion of subjects with abnormal ALAT values at baseline (non-clinically significant elevations acceptable at investigator's discretion).
2. Exclusion of participants with medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months
3. Investigator oversight of use of medications for symptomatic relief, including acetaminophen.

Other known risks associated with CHMI are discussed in section 6.3.

2. OBJECTIVES

Primary objectives:

1) Primary safety objective: To evaluate the safety and tolerability of the CHMI-transmission model in healthy Malian adult participants inoculated intravenously with either sporozoites (PfSPZ Challenge) or asexual blood-stages of *Plasmodium falciparum* (IBSM Challenge).
2) Primary efficacy objective: To determine the prevalence and kinetics of gametocytemia in healthy Malian adult participants inoculated intravenously with either sporozoites (PfSPZ Challenge) or asexual blood-stages of *Plasmodium falciparum* (IBSM Challenge).

Secondary objectives:

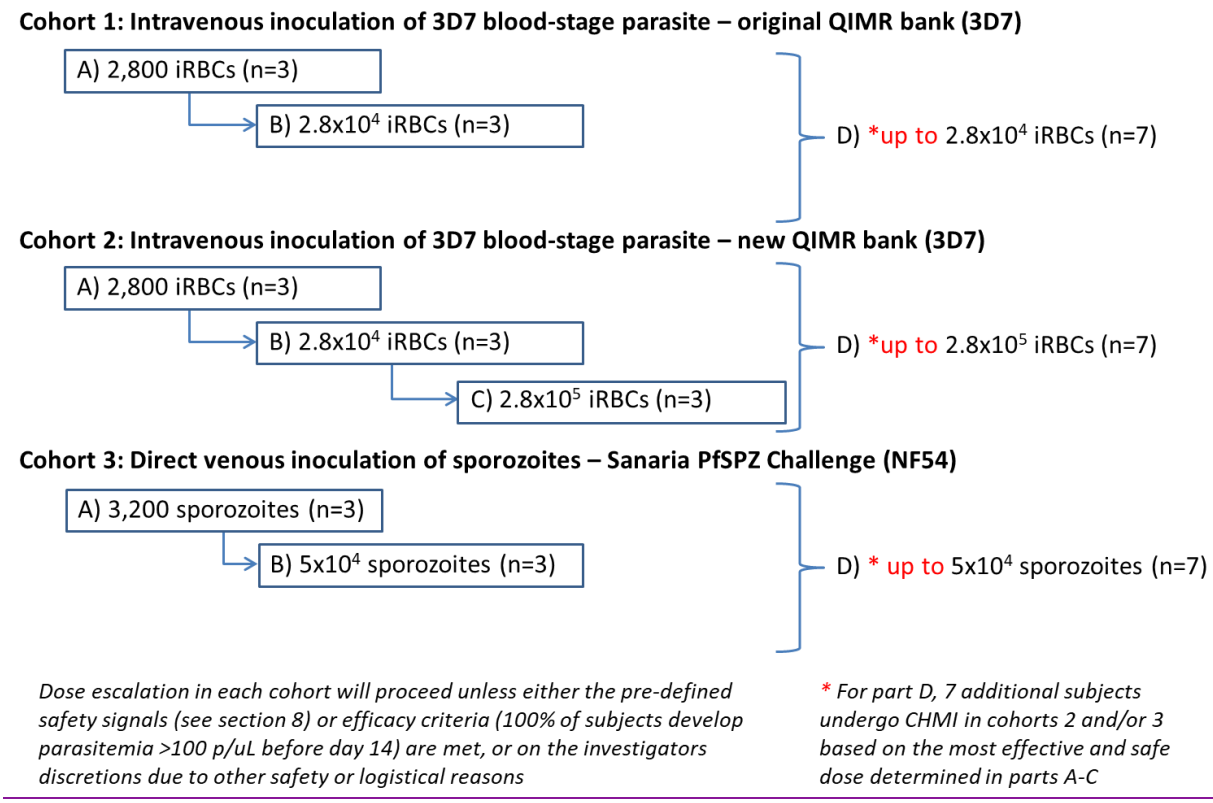
1) To determine the prevalence and kinetics of parasitemia in healthy Malian adult participants inoculated intravenously with either sporozoites (PfSPZ Challenge) or asexual blood-stages of *Plasmodium falciparum* (IBSM Challenge).
2) To assess the infectiousness of participants to *Anopheles* mosquitoes through Direct Skin Feeding Assay (DFA) and/or Direct Membrane Feeding Assay (DMFA).

Exploratory Objectives:

1) To determine the optimal parameters for the CHMI-transmission model in this population (inoculum type and dose, and timing of mosquito feeds), based on parasite/gametocyte kinetics and provisional DFA/DMFA results
2) To determine the required sample size for a CHMI-transmission study to evaluate efficacy of transmission-blocking interventions
3) To assess the dynamics of gametocyte commitment, maturation and gametocyte sex ratio.
4) To assess transmission reducing activity (TRA) of participants' sera at baseline in Standard Membrane Feeding Assays (SMFAs) and assess correlations with DFA/DMFA during CHMI-transmission model
5) To assess correlations between baseline markers of malaria-exposure and asexual and sexual stage immunity with parasite and gametocyte kinetics during the CHMI-transmission model
6) To analyse immune responses in participants inoculated intravenously with either sporozoites (PfSPZ Challenge) or asexual blood-stages of *Plasmodium falciparum*

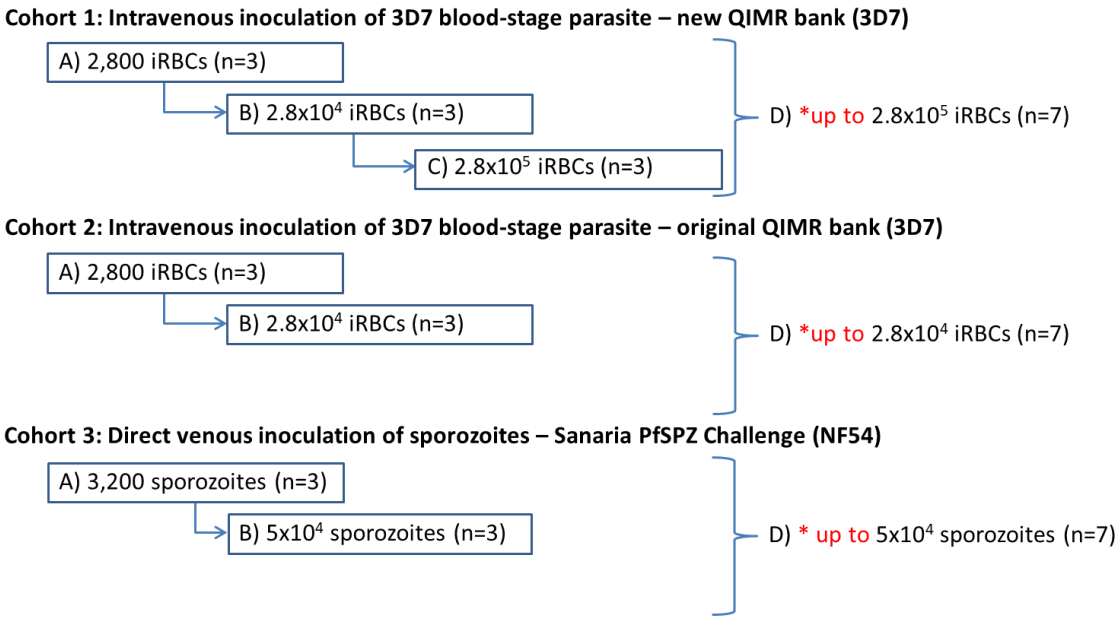
3. STUDY DESIGN

The study will be a single center, open label, clinical trial. A maximum of 42 healthy male participants aged 18 to 50 years will be recruited following screening ~~and serological profiling for low levels of cumulative malaria exposure [7]~~. The gender and age range are chosen to maximize homogeneity in the population and to rule out pregnancy during the study, due to the uncertain (albeit extremely low) risk of developing anti-RBC alloantibodies (see section 6.2). Participants will be assigned to one of the (sub-)cohorts:



Dose escalation in each cohort will proceed unless either the pre-defined safety signals (see section 8) or efficacy criteria (100% of subjects develop parasitemia >100 p/uL before day 14) are met, or on the investigators discretions due to other safety or logistical reasons

** For part D, 7 additional subjects undergo CHMI in cohorts 2 and/or 3 based on the most effective and safe dose determined in parts A-C*



Participants will be confirmed parasite free by qPCR 1 to 3 days before challenge. If a planned participant (screened and eligible for participation) is subsequently unable to participate or becomes ineligible before challenge on day 0, he will be replaced by another participant provisionally enrolled at the inclusion visit. For this purpose, two to four additional screened participants will be provisionally enrolled and invited to the challenge visit to act as back-ups.

Participants in **Cohort 1 and 2** will undergo controlled human malaria infection (CHMI) through a standard blood-stage challenge with *Pf*-infected erythrocytes by intravenous injection. Participants in **Cohort 3** will undergo a CHMI through a standard sporozoite challenge with PfSPZ Challenge (NF54) by direct venous inoculation (Figure 2). All cohorts will include the potential for dose escalation, and in principle the dose will increase 10/20-fold between parts (A, B and where applicable C). The starting inoculum dose will be that which has proven to be safe in previous CHMI studies. For the QIMR blood-stage inoculums this is ~2,800 viable parasites in malaria-naïve participants [5, 6] [8]. For PfSPZ Challenge this is 3,200 sporozoites both in malaria-naïve participants (clinicaltrials.gov: NCT01624961 [9]) and pre-exposed adults in Mali [10]. Each cohort may also include a consolidatory part (D), using the most effective and safe dose determined in parts A-C. Parts A, B, (C) and D for each cohort will be conducted sequentially, a minimum of 14 days apart. For logistical reasons, Cohorts 1, 2 and 3 will not necessarily be conducted together. Dose escalation within each cohort (from part A to B and where applicable from B to C) will proceed if the efficacy criterion (detection of ≥100 p/µL in all subjects before day 14) is not met in the preceding part of that cohort, and in the absence of protocol-defined safety signals up to day 14 (any subject with a grade 3 AE probably or definitely related to CHMI; and/or >2 subjects with a grade 2 AE probably or definitely related to CHMI; and/or any subject with parasitemia ≥100 p/µL upon first detection). If any such safety signals are observed, ~~an SMC~~a DSMB meeting will be convened to determine if it is safe to continue with dose escalation in that cohort. If an efficacious dose is identified for Cohort 1, 2 or 3 before completing the full dose escalation schedule, the Principal Investigator may elect to challenge all the remaining participants in that cohort with efficacious dose, along with the participants in part D. Based on data from Parts A and/or B, ~~an SMC~~a DSMB meeting may also be convened to adjust the dose escalation schedule outside of the planned 10-20 fold dose increases (up to maximally 100-fold

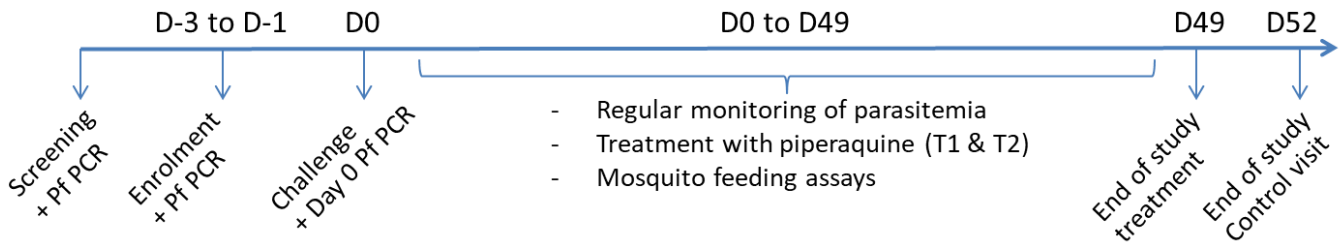
dose escalation at each step). The Principal Investigator may moreover decide to not conduct further dose-escalation and/or consolidation within any individual cohort due to logistical constraints.

After CHMI, participants will be closely followed with regular visits to the clinical trial centre (once daily until parasitemia reaches 1000 parasites/µL: Figure 2). Treatment with piperaquine will be used when necessary to clear pathogenic asexual parasites whilst leaving gametocytes unaffected. Treatment with low dose piperaquine (T1, 480 mg) will be initiated when either; (a) parasitemia reaches 1000 p/µL (in line with other CHMI studies in Africa) [11, 12], or (b) if a participant develops signs or symptoms of malaria accompanied by a thick-film blood smear with any density of asexual *Plasmodium* parasites. The initial clearance of parasitemia will be carefully monitored using daily blood samples. In case of clinical treatment failure or if recrudescent asexual parasitemia ≥1000 p/µL develops, a second, higher dose of piperaquine (T2, 960mg) will be administered. These treatment regimens cure asexual parasitemia (the pathogenic stage that causes symptoms) while leaving immature and mature gametocytes unaffected [13]. Clinical decisions will be based on duplicate microscopy readings. If a participant experiences symptoms of malaria while asexual parasites are being cleared, anti-inflammatory medications such as ibuprofen or paracetamol may be administered for comfort (Appendix 1). Tolerance of mild symptoms under supervision while asexual parasites are being cleared allows for the development of sufficient gametocytemia for parasite transmission.

Microscopic and/or molecular analysis of asexual parasite and gametocyte growth profiles will identify the optimal timing for mosquito feeding. Venous blood samples will be collected for Direct Membrane Feeding Assays (DMFAs) and/or Direct Skin Feeding Assays (DFAs) per MRTC SOPs on up to 3 occasions, the exact time-point being based on the density of gametocytemia as measured by gametocyte qRT-PCR and/or microscopy. These assays will provide evidence of the infectivity of participants at these time-points.

On day 49 (end of study) or if a second recrudescence ≥1000 p/µL occurs or if there is a safety concern or adverse event that in the opinion of the safety monitor or investigator requires immediate curative antimalarial treatment, participants will receive end of study treatment (artemether/lumefantrine and low dose primaquine) to ensure they are parasite and gametocyte free. For those participants who remain parasite negative by TBS for 19 days after blood-stage challenge, or for 21 days after sporozoite challenge, the frequency of follow-up visits will be reduced to ~3x/week; for any individuals who remain negative by PCR until 26 days after blood-stage challenge or until 28 days after sporozoite challenge, end of study treatment will be given, and end of study will apply for that participant.

The challenge study will take place in a setting designed to minimise exposure to local mosquitoes (in the dry season in an area with very low transmission) and participants will be required to remain in the study area for the duration of CHMI follow-up. Participants should have access to a mobile phone upon which they can be reached 24 hours per day and 7 days per week. Surveillance data suggest that *Anopheles sp.* mosquitoes, which are capable of transmitting malaria, represent a small proportion of overall mosquitoes in the study area (even during the wet season), and historical inoculation rates are low [60]. As additional safety precautions, participants will be required to stay in appropriate accommodation, use insect repellant as directed, and sleep under new insecticide-treated nets during the study period to reduce any risk of mosquito bites in the community. This is to remove the risk of exposure to local mosquitoes. Study staff may perform home visits to ensure accommodation is appropriate, and all participants must demonstrate an understanding of these requirements prior to enrolment



Screening: eligible subjects screened with a medical history, physical examination, vital signs, ECG, laboratory evaluations, *P. falciparum* qPCR and serology to determine level of pre-exposure to malaria
Enrolment: eligible subjects enrolled and *P. falciparum* qPCR performed
Monitoring of parasitemia: parasitemia and gametocytemia will be monitored daily from Day 5 after challenge using TBS and/or qPCR. Frequency will be reduced to ~3xper week if i) the participant remains parasite negative by TBS 21 days following challenge, or ii) if the participant remains parasite negative by TBS for 7 days following T1 or T2
Piperaquine treatment: subjects will be treated with piperaquine 480mg (T1) when parasitemia reaches 1000p/µl. If recrudescent asexual parasitemia ≥1000p/µl develops, piperaquine 960mg (T2) will be administered.
Mosquito feeding assays: direct membrane feeding assays (DMFA) and direct skin feeding assays (DFA) performed on up to 3 occasions when gametocytes are present
End of study treatment: at day 49, or if i) a second recrudescence of asexual parasitemia develops following T2, or ii) a subject remains 18S qPCR-negative at day 28 after challenge, the subject will receive final end of study treatment (T3, artemether/lumefantrine and primaquine)

Figure 2 – CHMI-trans Mali study design

Data from previous CHMI studies in malaria naïve participants indicate that after the initial challenge the first possible moment of parasitemia to be at day 6 (sporozoite challenge) or day 4 (blood-stage challenge). Participants will be monitored daily from day ~~6 (sporozoite challenge) or day 4 (blood-stage challenge)~~5 onwards until malaria parasites are detected at ≥1000 p/µL, or asexual parasitaemia of any density in the TBS in combination with symptoms or signs of malaria, upon which they are treated with piperaquine 480mg (T1). If a positive recrudescence of asexual parasitemia ≥1000 parasites/µL or clinical treatment failure occurs after T1, subjects will receive piperaquine 960mg (T2). On day 49 or if a second TBS positive asexual parasite recrudescence ≥1000 p/µL or clinical treatment failure occurs after T2, or if there is a safety concern or adverse event that in the opinion of the safety monitor or investigator requires immediate curative antimalarial treatment, participants will receive a curative regimen (T3, A/L+PQ) for radical clearance of all parasite stages.

4. STUDY POPULATION

4.1. Population (base)

The study population will be comprised of healthy adult males aged 18 to 50 years; women will not be included in this current study due to the uncertain (albeit extremely low) risk of developing anti-RBC alloantibodies (see section 6.2). A maximum of 42 participants will be enrolled in the study and will be assigned to one of the cohorts. 2-4 additional ~~participants~~ screened individuals per cohort may be ~~screened and~~ invited to the inclusion visit as back-ups. Unused back-ups may be invited to participate in later cohorts if they continue to meet eligibility criteria. The investigator will ensure that all subjects being considered for the study meet the following eligibility criteria. Participant eligibility is to be established and confirmed by checking through all inclusion/exclusion criteria at both screening and baseline. A relevant record (e.g. checklist) of the eligibility criteria will be stored with the source documentation at the study site.

The study will be conducted at MRTC Sotuba. Sotuba is a community located on the outskirts of Bamako on the bank of the Niger River, population ~7,000. Malaria transmission peak occurs from August to November. The entomological inoculation rates are historically low. The annual rainfall varies between 800 mm and 1000 mm and occurs from June to October. Cumulative malaria exposure in Sotuba is modest compared to highly endemic parts of Mali. Among 268 patients attending for care in Sotuba with symptomatic malaria between 2017 and 2019, the mean parasitemia at time of presentation was 10268p/uL (range 40 to 233600) (Unpublished data, MS Sissoko). Many clinical trials (malaria vaccine and drug trials), as well as epidemiological and entomologic malaria studies, have been conducted in Sotuba.

4.2. Inclusion criteria

In order to be eligible to participate in this study, a participant must meet all of the following criteria:

1. Males aged ≥ 18 and ≤ 50 years and in general good health.
2. Known long-term resident (more than 1 year) of Sotuba or surrounding area.
3. Participant has adequate understanding of the procedures of the study and is able and willing (in the investigator's opinion) to comply with all study requirements, including, but not limited to:
    a. remaining in Sotuba during the challenge period, not travelling during the study period, and remaining reachable (24/7) by mobile telephone throughout the entire study period
    b. available to attend all study visits, and willing to sleep in appropriate accommodation close to the trial center during part of the study (from day 5 post-infection until either (i) end of study, or (ii) day ~~26/~~28 if parasitemia does not develop before this time
    c. refraining from blood donation throughout the study period and for a 6 week period thereafter
4. Able to provide proof of identity to the satisfaction of the study clinician completing the enrolment process.
5. Willing to have blood samples stored for future research.
6. The participant has correctly answered ≥80% of the questions on the Study Comprehension Exam.

4.3. Exclusion criteria

A potential participant who meets any of the following criteria will be excluded from participation in this study:

1. Any history, or evidence at screening, of clinically significant symptoms, physical signs or abnormal laboratory values suggestive of systemic conditions, such as cardiovascular, pulmonary, renal, hepatic, neurological, dermatological, endocrine, malignant, haematological, infectious, immunodeficient, psychiatric and other disorders, which could compromise the health

of the participant during the study or interfere with the interpretation of the study results. These include, but are not limited to, any of the following.

1.1. Body weight <50 kg or Body Mass Index (BMI) <18 or >30 kg/m2 at screening.
1.2. History, or evidence at screening, of elevated risk for cardiovascular disease, including arrhythmia or clinically relevant bradycardia, prolonged QT-interval (>450ms) or other relevant ECG abnormalities; a positive family history of cardiac events in 1st or 2nd degree relatives <50 years old, or of sudden (cardiac) death.
1.3. Severe asthma, defined as asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past 2 years, or that has required the use of oral or parenteral corticosteroids at any time during the past 2 years
1.4. History of a severe allergic reaction or anaphylaxis
1.5. Autoimmune or antibody-mediated disease including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, or autoimmune thrombocytopenia
1.6. A medical history of functional asplenia, sickle cell disease, thalassaemia trait/disease or G6PD-deficiency.
1.7. History of epilepsy in the period of five years prior to study onset, even if no longer on medication.
1.8. Screening tests positive for Human Immunodeficiency Virus (HIV), active Hepatitis B Virus (HBV), Hepatitis C Virus (HCV)
1.9. Hemoglobin, white blood cell (WBC), absolute neutrophil count, or platelet, alanine transaminase (ALT) or creatinine (Cr) levels outside the local laboratory–defined limits of normal. (Subjects may be included at the investigator's discretion for "not clinically significant" values outside of normal range and ≤ Grade 2.
1.10. Chronic use of immunosuppressive or other immune modifying drugs within three months prior to study onset (inhaled, intranasal and topical corticosteroids and oral anti-histamines exempted) or expected use of such during the study period.
1.11. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years.
1.12. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the participant to understand and comply with the study protocol
1.13. Suspicion of alcohol or illicit drug abuse interfering with health or normal occupational or social function in the period of one year prior to study onset.

2. Any recent or current systemic therapy with an antibiotic or drug with potential anti-malarial activity (chloroquine, doxycycline, tetracycline, piperaquine, benzodiazepine, flunarizine, fluoxetine, tetracycline, azithromycin, clindamycin, erythromycin, hydroxychloroquine, etc.; allowable timeframe for use at the Investigator's discretion).
3. Previous receipt of any malaria vaccine unless approved by the Principal Investigator.
4. Receipt of any live (attenuated) vaccine within the past 4 weeks, or of any vaccine within the past 2 weeks of enrolment
5. Known hypersensitivity to or contra-indications (including co-medication) for use of piperaquine, artemether-lumefantrine, primaquine, latex or history of severe (allergic) reactions to mosquito bites.
6. Current use of any drug that is metabolised by the cytochrome enzyme CYP2D6 (e.g. flecainide, metoprolol, imipramine, amitriptyline, clomipramine).
7. Current use of drugs that are known to prolong the QTc interval such as: antiarrhythmics of classes I and III; neuroleptics and antidepressant agents; certain antimicrobials including some macrolides, fluoroquinolones, pentamidine, saquinavir, imidazole, and triazole antifungal agents; certain non-sedating antihistaminics (terfenadine, astemizole); cisapride.
8. Use of immunoglobulin or blood products within 6 months prior to enrolment

9. Participation in any other clinical study, or receipt of any investigational product, in the 30 days prior to the start of the study or during the study period unless approved by the Principal Investigator.
10. Any other significant disease, disorder or finding which, in the opinion of the investigator, may significantly increase the risk to the participant because of participation in the study, affect the ability of the participant to participate in the study or impair interpretation of the study data. In case participants are excluded due to diagnosis of a contra-indication during screening, the study will ensure and cover treatment for acute conditions and referral for chronic conditions.
11. For cohort 1 and 2 (blood stage challenge): Known receipt of a blood transfusion in the past.

5. SAMPLE SIZE CALCULATION

This study is designed to establish a CHMI-transmission model in pre-exposed Malian adults, by assessing gametocyte prevalence and clinical symptoms during CHMI induced by either PfSPZ Challenge or blood-stage inoculation. We will determine the suitability of inoculum type and dose to induce gametocytemia, defined as gametocyte prevalence by gametocyte qRT-PCR on any moment during follow-up. Based on preliminary data from a study in The Gambia, after selecting individuals based on their low response to serological markers of malaria exposure, we expect >95% individuals will develop asexual parasitemia. In the CHMI study in malaria exposed individuals in The Gambia, 100% (10/10) of those with low sero-reactivity developed asexual parasitemia greater than 5p/µL within 15 days of challenge [7]. Based on data from the CHMI-transmission studies in malaria naïve individuals, we expect that 100% of those who develop asexual parasitemia >5p/µL will develop gametocytemia ~1-2 weeks later [5, 6]. The CHMI-transmission approach is considered unsuitable if <50% of individuals develop mature gametocytes; the lower limit of the confidence interval around the proportion of gametocyte positive individuals should thus be above 50%. For each inoculum we will evaluate the optimal dose in a total of 10 individuals, 9 of whom should thus become gametocyte positive, the lower limit of the 95% confidence interval around this proportion is 55%.

Comparisons between groups are underpowered by conventional frequentist approaches and a Bayesian model will be used to select the optimal challenge type and dose for future CHMI-transmission studies with evidence for highest gametocyte prevalence and density.

6. CONTROLLED HUMAN MALARIA INFECTIONS

6.1. Description of materials and procedures used to induce CHMI

In this study CHMI will be induced by intravenous inoculation of either *P. falciparum*-infected erythrocytes (3D7 clone) or purified, aseptic, cryopreserved, infectious *P. falciparum* sporozoites (NF54 strain). The NF54 strain of *P. falciparum* was isolated from a patient living in the Schiphol-area in the Netherlands and has been shown genetically to originate from West Africa. The 3D7 parasite is a clone of NF54. In *in vitro* studies, both have been shown to be completely susceptible to multiple antimalarials, including chloroquine, mefloquine, piperaquine, sulfadoxine-pyrimethamine, atovaquone/proguanil and artemether/ lumefantrine (see respective Investigator's Brochures).

6.1.1. *P. falciparum*-infected erythrocytes (3D7 clone)

In this trial, two banks of infected ***P. falciparum*-infected erythrocytes (3D7 clone)** will be used for intravenous injection (Cohorts 1 and 2). Both banks originate from the Queensland Institute of Medical Research (QIMR), Brisbane, Australia, see also the Investigator's Brochure:

- **original QIMR 3D7 bank (3D7-V2)**
- **new QIMR 3D7 bank (3D7-MBE-008)**

The initial bank was derived from blood donated by a participant undergoing CHMI with the *Plasmodium falciparum* strain 3D7 by mosquito bite. The preparation of this challenge inoculum has been described in detail [40, 42], briefly: Before the CHMI, the donor was extensively screened and no serological evidence was found for infectious agents including Hepatitis B, HIV, HCV, Human T-lymphotropic Virus (HTLV) 1+2 or syphilis, with exception of seropositivity for Epstein-Barr virus and cytomegalovirus (However, the stored blood sample is PCR negative for both viruses, indicating absence of viral DNA). Once the donor was microscopically positive for presence of asexual blood-stage *P. falciparum* parasites, one unit of blood (500 ml) was collected from the donor and processed to remove leucocytes. The packed blood cells were then mixed with glycerolyte 57 solution (Baxter, Deerfield, IL) and cryopreserved in ~1 mL aliquots as previously described [40, 42] and stored at QIMR Berghofer under controlled conditions. The bank has tested negative by culture for bacteria and fungi and for *Mycoplasma* contamination. Aliquots are thawed and administered to participants by intravenous inoculation. The new bank was created by *in vitro* expansion of material from the origin bank using a bioreactor [8, 61]. Closed system culture bioreactor expansion using clinical grade leukodepleted packed red blood cells has been used previously to develop other parasite cell banks for use in human challenge, including genetically modified and artemisinin-resistant strains [61, 62]. This method of culture is cost-effective and reduces the need for human passage. This new bank was created under GMP conditions using the original sensitive 3D7 strain to produce new stock to maintain supply for ongoing IBSM challenge studies [8]. The protocol for manufacture and release of such banks has been favorably reviewed by the US FDA (manuscript describing this under review).

The infective inoculum is prepared from a single aliquot of the respective cryopreserved infected packed blood cells prepared as previously described [42] (see also Investigator's Brochures for detailed description). Each dose of 2 mL will contain approximately the stated number of viable parasite infected erythrocytes. The inoculum will be prepared aseptically, as outlined in Standard Operating Procedures documents.

Thawing and washing of the inoculum will be done with commercial cGCP solutions for human use and with disposable syringes and needles according to standard operating procedures used in previous studies at QIMR and Radboudumc. Sample manipulations will be performed within a safety cabinet that has been especially cleaned and set aside for this purpose. After thawing of the blood inoculum, the cold chain (2-8 degrees) will be maintained at all times until the inoculum has been administered to the participant. Each step in this process is performed by a trained technician and

checked and recorded on standardized forms by another technician according to standard operating procedures. Following preparation, the inoculum will be stored on ice and administered intravenously to participants within the timeframe outlined in the IB.

The inoculum will be administered by intravenous injection into an indwelling intravenous cannula. The approximate number of infected red blood cells will be injected in a total volume of 2 mL of normal saline followed by a saline flush. The cannula will then be removed, and hemostasis ensured by use of an appropriate dressing.

6.1.2. PfSPZ Challenge (NF54)

Cohort 3 participants will receive PfSPZ Challenge (NF54) [a suspension of purified, aseptic, cryopreserved, infectious *P. falciparum* sporozoites (NF54 strain)] by direct venous inoculation, produced by Sanaria, Inc., Rockville, MD, U.S.A.), see also the Investigator Brochure (v 16.0, 03 May 2021):

PfSPZ Challenge (NF54) is thawed and formulated in diluent on the day of administration. The cryopreserverd sporozoites are prepared as described in the investigator brochure. Briefly, aseptically-reared female *Anopheles* mosquitoes are fed on *in vitro* cultured *P. falciparum* gametocytes (NF54 strain) under GMP conditions. Once sporozoites develop, these are dissected from the salivary glands and purified. Batches of PfSPZ Challenge are dispensed in a 0.5 mL screw-cap vial (Matrix TrakMates) containing PfSPZ. The vials are stored in liquid nitrogen vapor phase (-140° to -196°C).

Supplies of cryovials containing PfSPZ Challenge will be provided to the study site by Sanaria Inc. Qualified study personnel will prepare each dose as described in the relevant SOPs just prior to administration. Specifically, SOP 336 describes the method for preparation of PfSPZ Challenge and a linked form. The form linked to this SOP includes a checklist for the equipment and supplies required for the preparation of the dose concentration. The written instructions for preparation of the dose concentration will be authorized by Sanaria QA. PfSPZ will be shipped in a liquid nitrogen vapor phase (LNVP) shipper that also holds a temperature data logger as well as a temperature probe. PfSPZ is stored in the shipper itself and must not leave the shipper. The LNVP shipper is the size of a biohazard trash can (about 2.5ft x 2 ft). The data logger captures the temperature of the inside of the shipper where PfSPZ resides every 5 minutes from the time it leaves Sanaria, while it is on the airplane, when it arrives in Mali and while it sits waiting at the clinical site until one decides to stop. The data logger can be downloaded on any computer to observe the temperature trace. The temperature probe allows an immediate temperature check. No administration is allowed or done in case of (partial) thawing or temperatures outside the permitted range. There are strict SOPs that an Operator is trained to and documents that record all steps that have to be followed.

At the clinical site, the preparation of all doses of PfSPZ Challenge material, from start to finish, will be monitored and inspected by a qualified person. Each form will have a requirement for sign-off by the qualified person.

Each inoculation will be administered by the co-investigator, clinical investigator, or a study nurse as described in the relevant SOP. Inoculations will be administered as injections, each containing the same volume. Participants will receive the injections IV.

6.2. Summary of findings from clinical studies

CHMIs are well accepted as a powerful tool for the evaluation of parasite development in humans. Since 1986 thousands of participants worldwide have undergone a CHMI by the bites of *P. falciparum*-infected mosquitoes, by inoculation of *P. falciparum* infected-erythrocytes, or by inoculation of purified, aseptic, cryopreserved, infectious *P. falciparum* sporozoites (PfSPZ Challenge) [40, 42]. These have proven to be reproducible, predictable and generally safe methods of inducing

P. falciparum malaria. The results of such studies were summarized in 1997 [23], in 2007 [48] and in 2012 [63], and in 2018 [38]. Most CHMI studies worldwide are conducted with either the NF54 strain of *P. falciparum*, or its derivative clone 3D7.

6.2.1. *P. falciparum*-infected erythrocytes (3D7 clone)

See also the Investigator's Brochure. The original *Plasmodium falciparum* 3D7 parasite bank for blood stage challenge has been used to safely inoculate over 400 malaria naïve study participants in previous challenge studies [63][64]. The new QIMR bank has been administered to 2 malaria naïve participants where it demonstrated similar safety and infectivity to the original bank [8]. A single SAE related to the challenge inoculum has been reported in one of these studies [65]. In this recent study, one case of severe neutropenia related to the malaria challenge agent was recorded as an SAE. The participant was inoculated with *Plasmodium falciparum* 3D7 and recorded a neutrophil count of 0.10 × 10^9/L (Normal range: 1.5 to 6.5 × 10^9/L) prior to being dosed with the study drug on Day 8. An independent haematologist concluded that the observed neutrophil count decrease was likely a consequence of the malaria infection itself. Neither the original bank nor the new bank has previously been administered to previously-exposed African adults.

The time range to blood stage parasitemia detectable by PCR (prepatent period) in malaria naïve adults is reliably between 3 and 4 days. Mild-moderate solicited adverse events are generally experienced by all study subjects, most commonly headache, general malaise/fatigue and fever [49]. Gastro-intestinal complaints, including nausea, diarrhea and abdominal pain have also been reported, mainly following intake of piperaquine or atovaquone-proguanil.

6.2.2. PfSPZ Challenge (NF54)

See also the Investigator Brochure (v 16.0, 03 May 2021). PfSPZ Challenge (NF54) has been administered to well over 1000 subjects, including over 550 previously exposed African adults, across >35 trials in 11 (including 6 African) countries. Administration is safe and extremely well tolerated. The most common adverse events are in fitting with early malaria infection and when they occur are generally mild to moderate. In malaria naïve adults, inoculation of the standard dose of 3,200 PfSPZ invariably results in parasitaemia and malaria symptoms. Amongst prior-exposed African adults, however, the proportion of participants developing parasitaemia, and the proportion developing symptoms of malaria, vary between studies, likely reflecting differences in pre-existing immunity. E.g. in two previous CHMI studies in Gabon, 9/54 (17%) participants remained sterilely protected, whereas 23 (43%) developed parasitaemia but managed to control this without developing symptoms and 22 (40%) developed an episode of malaria and required treatment [11, 12]. In an ongoing study, 6/28 (21%) remained sterilely protected, whereas 20 (71%) developed and controlled parasitaemia and 2 (6%) developed malaria. No product-related SAEs have occurred.

6.3. Summary of known and potential risks and benefits

Please see also the Investigator's Brochures. There is no direct benefit expected for subjects participating in this study. The risk to subjects after challenge infection with 3D7-infected human erythrocytes or PfSPZ Challenge are largely similar and will discussed together. Potential risks associated with undergoing CHMI include i) discomfort or bruising due to intravenous inoculation of the challenge inoculum, ii) discomfort or bruising associated with periodic blood drawing and iii) risk of acquiring mild clinical *P. falciparum* malaria.

Direct venous inoculation of both *P. falciparum*-infected erythrocytes and PfSPZ Challenge are generally tolerated extremely well and will be performed by experienced trained staff. Any bruising caused by inoculation or blood draw will be managed symptomatically. The total amount of blood collected will be maximally 500 ml (i.e. the volume normally collected from blood donors) over the entire trial period.

Symptoms of mild malaria (e.g. headache, myalgia, fever, etc.) tend to be of short duration following start of antimalarials and will be treated symptomatically with paracetamol or other appropriate medication.

The risk of developing manifestations of severe malaria will be minimized by adherence to the inclusion/exclusion criteria and close clinical monitoring, which ensures that subjects with malaria will be treated at earliest stages of parasitemia. Moreover, participants are expected to have some level of acquired immunity to malaria through natural exposure, further limiting the risk of developing high parasitemia and/or severe disease.

As discussed in section 1.4, several cases of cardiac SAEs have occurred during CHMI studies in Nijmegen, the Netherlands. Although neither the aetiology nor pathophysiology are fully elucidated, these episodes have the following characteristics:

- All occurred following mosquito bite CHMI. (No cases have been recorded in Nijmegen or elsewhere following either PfSPZ Challenge or *P. falciparum*-infected erythrocyte CHMIs).
- All occurred several days after initiating antimalarial treatment, when parasitaemia was no longer detectable and symptoms had generally subsided.
- Cases occurred following infection with both NF54 and NF135 strains and following treatment with a range of antimalarial drugs (artemether/lumefantrine, atovaquone/proguanil, chloroquine)
- Cardiac imaging tends to suggest myocarditis, although this was not evident in all cases.
- All cases resolved clinically without intervention, although in some cases residual scarring was observed by MRI

The risk of a cardiac SAE in our study is considered very small, as no cases have been recorded elsewhere following mosquito CHMI, or in either Nijmegen or elsewhere following PfSPZ Challenge (NF54) CHMIs. Following *P. falciparum*-infected erythrocyte CHMI only non-serious transient cardiac adverse events (ventricular extrasystoles attributable to unmasking of a predisposition to benign fever-induced tachyarrhythmia) have very occasionally occurred. This risk will be further reduced by strictly adhering to In- and Exclusion criteria. Participants complaining of chest pain during the study will undergo an ECG and be evaluated clinically by appropriately-trained medical staff.

Asymptomatic liver function test abnormalities involving self-limited transaminase elevations have been reported in malaria-naïve CHMI participants following PfSPZ, mosquito-bite, and IBSM challenge [56, 57. While the majority of these adverse events are minor, and similar to those experienced following natural malaria infection, grade 4 elevations in alanine transferase (ALT) have been observed in up to 5% of participants following IBSM challenge [57]. These transient biochemical adverse events typically occur in the 3 to 10 days following administration of antimalarial treatment, are not associated with elevated bilirubin, and may be associated with high asexual parasitemia and cumulative paracetamol (acetaminophen) exposure. These elevations have been observed following multiple classes of antimalarial drug, and are thought related to inflammation associated to parasite clearance rather than drug-associated toxicity. Compared to malaria naïve individuals, liver function test abnormalities following natural malaria infection are less common in malaria-experienced individuals [58, 59] and these adverse events appear to be less common following CHMI in malaria endemic areas (R. Sauerwein, personal correspondence). The risk of asymptomatic liver function test abnormalities is considered lower for a Malian CHMI study population compared to a malaria naïve study population. To further understand and quantify this risk active monitoring for biochemical abnormalities is included in the safety sample collection schedule. Risk will be reduced by strictly adhering to in- and exclusion criteria.

Specifically for subjects receiving *P. falciparum*-infected erythrocytes (cohorts 1 and 2), there is a theoretical that subjects could suffer a transfusion reaction after they receive the inoculum, or

develop alloantibodies to the donor RBCs that may make blood transfusion more difficult in the future. However, this risk is considered extremely low since the donor blood used to produce the inocula for the original and new QIMR banks was blood group O Rh(D) Negative. People with this blood group are generally considered “universal donors”, as recipients of their blood have minimal risk of developing RBC alloantibodies when given much larger volumes of blood than is used in the blood stage challenge model. To date, one subject has developed an antibody response to a minor Rh antigen (anti-E antibody) following blood stage inoculation with P. falciparum 3D7 (see Investigator Brochure). However, there was no laboratory evidence to indicate that the specific Rh phenotype of the donor RBCs in the inoculum provoked production of this allo anti-E antibody. Participants will be monitored for signs and symptoms in the period immediately after administration of the inoculum to further assess the risk of the inoculum in causing transfusion reaction. Participants ~~of cohort 1 and 2~~ will also be tested for RBC alloantibodies at screening and at the end of the study as part of their safety monitoring.

Pregnant women are at higher risk of developing severe malaria. Furthermore, women of childbearing potential have a small additional risk of developing RBC alloantibodies from erythrocyte inoculations that could cause problems during subsequent pregnancies. For both these reasons, only male participants will be included in this pilot study. Development of RBC alloantibodies will be evaluated in the participants of the current study, and the data used to review the risk of including women in future CHMI transmission studies using this protocol.

7. CO-INTERVENTIONS

7.1. Use of antimalarial medication

Various (non-investigational) antimalarial drugs will be used in the course of this study for specific purposes:

- **Piperaquine**: clearance of first wave of asexual parasitaemia (T1) and first recrudescence (T2) following CHMI
- **Artemether/lumefantrine**: final treatment (following second recrudescence or at end of study)
- **Primaquine:** supplementary final treatment in combination with A/L, in order to clear residual circulating gametocyte

Various (non-investigational) over the counter medications may be used for symptomatic relief in participants at the discretion of the investigator (e.g. paracetamol, ibuprofen). These medications are to be used under the supervision of study staff.

7.2. Piperaquine

Upon reaching a pre-defined parasitemia threshold following CHMI, oral piperaquine will initially be administered as a single sub-curative oral dose (480mg), intended to induce partial clearance of asexual parasites followed by recrudescence. This is thought to cause an increase in asexual commitment to gametocytes through terminal investment of malaria parasites under drug pressure [13, 19] [20]. If recrudescence occurs after 480mg piperaquine, participants will subsequently receive a higher oral dose (960 mg) to clear recrudescent parasitemia, without affecting (mature) gametocytes.

The antimalarial activity of piperaquine when administered as a single agent in a malaria challenge model has been established at QIMR Berghofer Medical Research Institute, Australia [66], in a dose finding study. Administered as a single dose (960, 640 and 480 mg) the drug rapidly cleared asexual parasitemia. In subsequent CHMI-transmission studies in malaria naïve individuals receiving low dose piperaquine (480mg), 12/17 and 10/24 had recrudescent infections which were completely cleared after a second, higher dose of piperaquine (960 mg) [5, 6].

Piperaquine has a very long terminal elimination half-life, 531 h (22 days) and 468 h (20 days) in adults and children, respectively [67]. Piperaquine is also highly lipophilic, and its oral bioavailability is approximately doubled by administration with a high-fat meal [68, 69]. It is well tolerated [67], with the main adverse events reported to be gastrointestinal disturbance such as diarrhea [70]. Electrocardiographic effects of piperaquine have been specifically evaluated in two studies [71-74]. Both demonstrated a prolongation of the corrected QT interval during treatment (between 11 and 14ms). Very few individual patients experienced a prolongation that could be regarded as clinically significant (>60ms), with a single instance of QTcF that exceeded 500 msec. Notably, QTc prolongation induced by piperaquine has not been associated with clinically relevant cardiovascular events suggesting a pro-arrhythmogenic effect. Therefore, although statistically significant, the QTc prolongation observed following piperaquine therapy is unlikely to be clinically relevant. European regulatory authorities have however recommended that DHA-PQP should not be administered with food (to reduce peak concentrations), and caution that prior and post electrocardiographic monitoring should be undertaken, as well as avoidance of concomitant consumption or recent exposure to drugs at risk of QTc prolongation [73, 74]. ~~The Malian National Malaria Control Program advises DHA-PQP should not be administered with food.~~ A large randomized clinical trial conducted in west Africa including Mali found treatment with DHA-PQP regardless of food intake was not associated with any adverse events [75].

The cardiac risk to participants in this trial will be minimized in the following ways:

- Potential participants with a history of cardiovascular disease or clinically significant ECG abnormalities will be excluded from participation in the study, with particular attention paid to cardiac conduction.
- ~~Treatment with piperaquine will be given after a fasting period of ≥ 3 hours in order to limit bioavailability and reduce risk of QTc prolongation. Subjects will be required to fast for a further four hours any time after dosing with piperaquine.~~
- Close clinical and laboratory monitoring cardiovascular effects after piperaquine treatment to ensure the safety and wellbeing of the healthy participants, including performing a 12-lead ECG timed to coincide with expected maximal piperaquine concentrations after oral dosing (within 4h-12h after treatment). Participants with increases in QT/QTc to >480 ms or >30 ms over baseline after initial piperaquine treatment (480mg) will not receive further dosing of piperaquine during the trial. These participants will instead receive alternative end of study treatment for T2, if required.

7.3. Artemether-lumefantrine

End of study medication consists of a standard curative adult oral course of artemether/lumefantrine as per National Malaria Treatment Guideline.

A/L is an extremely widely used, first line treatment for malaria, including in Mali. It is well tolerated and safe in the general population. Concomitant use of other medication that might cause interactions (in particular with lumefantrine), e.g. other antimalarials, will be screened for and avoided where possible or else monitored closely. The NF54 strain and 3D7 clone of *P. falciparum* are both known to be susceptible and no known treatment failures have occurred in CHMIs conducted with these parasites.

7.4. Primaquine

A single stat oral dose of low dose primaquine (0.25 mg/kg) will be part of the end of study medication, in order to clear gametocytes.

Adding primaquine at this dose is recommended by the WHO to prevent transmission and can be safely used in African patients regardless of the G6PD status [76].

7.5. Drug accountability

The principal investigator must ensure that non-investigational medicinal products are stored in an appropriate storage room. Accurate records must be maintained regarding the receipt of the treatments, which include: drug name, date received, lot number, amount received.
Accurate records must also be maintained regarding administration of drugs to participants. These records will be kept by the investigators. This includes:

- Participant identification number
- Date and dose of drugs dispensed
- Signature of the person administrating the drugs

The PI may delegate these responsibilities to appropriately qualified and trained pharmacy staff.

8. METHODS

8.1. Study parameters/endpoints

8.1.1. Primary study parameter/endpoints

- Safety: Frequency and magnitude of adverse events in each sub-cohort from challenge until end of study.
- Efficacy: Prevalence and density of gametocytes in each sub-cohort following challenge.

8.1.2. Secondary study parameters/endpoints

- Prevalence and density of parasitemia in each sub-cohort over time following challenge
- Proportion of infected *Anopheles* mosquitoes following DFA/DMFA in each cohort
- Intensity of oocyst infection in mosquitoes following DFA/DMFA in each cohort

8.1.3. Exploratory study parameters/endpoints

- The sample size required to evaluate the efficacy of transmission blocking interventions in this population determined through mathematical modeling of the parasite/gametocyte kinetics and DFA/DMFA results.
- Gametocyte commitment and maturation rates and gametocyte sex ratio determined by molecular markers of sexual stage development
- Transmission reducing activity of participants' baseline sera measured by reduction in prevalence of infected *Anopheles* mosquitoes and intensity of oocyst infection in SMFAs.
- Baseline serum levels against a panel of malaria antigens that may include (but is not limited to) AMA-1, MSP-1.19 and GLURP.R2, GEXP18, Rh2.2030, Etramp5.Ag1, Pfs230 and Pfs48/45, and/or malaria schizont extract.
- Induction of humoral and cellular immune responses by the CHMI-transmission model.

8.2. Study procedures

8.2.1. Recruitment and Screening

Community permission will be obtained from village elders, family heads, and other community members after explanation and discussion of the study [77]. The community permission process goes through the following steps:

- Study investigators/personnel explain the study to community leaders
- The community leaders then discuss the study with family heads and community members and relay any additional questions or concerns they may have to study personnel.
- The study and the informed consent process are explained in detail to individuals by study investigators/personnel.

The individual informed consent process and form will be translated into French. The study team will conduct careful word-for-word review of the study consent form and will translate the consent orally into local languages if potential study subjects do not read or speak French. Verification that the oral translations are accurate and that the potential subjects understand the contents of the informed consent form will be done by an independent witness who is not a member of the study team.

Participants who wish to participate in the trial will be asked to complete a study comprehension exam (Appendix 2). Their understanding of the trial will be tested after discussing the study with the investigator during informed consent, and after being asked to sign and date the consent form. Participants who fail to answer >80% questions correctly on their first attempt are allowed to re-take the questionnaire following further discussion of these issues with the investigator, and provided they subsequently achieve this score, they may then be screened for the trial.

Subjects who sign informed consent will undergo complete screening including a medical history, physical examination, vital signs, ECG and laboratory evaluations including thick blood smear and *P. falciparum* spp. qPCR. If physical examination, vital signs or laboratory values are out of the normal range a repeat measurement may be obtained and if the result remains out of range, clinical judgement will be applied as to whether the abnormality is clinically significant.

Participants found to be parasitaemic (by TBS and/or qPCR, any parasite density) ~~accompanied by symptoms of malaria,~~ will be treated as per Malian guidelines and will be excluded.

Although Pf infections are transient, a record of infection remains detectable in an individual's antibody profile with antibody responses decreasing over time [78-86]. Appropriately chosen antibody measurements using different Pf antigens can provide information about an individual's exposure history and this has been shown to provide an indication of the likelihood of becoming infected during CHMI [7]. To assess the rate at which potential trial participants were previously infected with Pf and thus their likelihood of becoming infected during CHMI and their suitability for inclusion in the study, we will assess serologic responses to ~~a panel of~~ Pf antigens ~~and/or schizont ELISA~~ during screening. Serology will be performed against ~~a panel of~~ malaria antigens that may include apical membrane antigen-1 (AMA-1), merozoite surface protein-1 (MSP-1) and glutamate-rich protein (GLURP) and RH2030, GexP and Etrap5.Ag1 and/or Pf schizont ELISA [7, 34]. Only subjects with the lowest serological responses in line with responses in those who were infected during a previous CHMI in pre-exposed adults in the Gambia will be eligible for this study. Screening may take place up to 120 days prior to challenge; if this timeframe is exceeded, the participant must be re-screened.

At the discretion of the investigator, the accommodation of the participant may be inspected and corrective actions taken to ensure that it is appropriate for participation. This may include mosquito spraying, or other environmental interventions to reduce the risk of community-based mosquito bites during the study.

Subjects who provisionally meet the eligibility criteria will be invited back for enrolment.

8.2.2. Enrolment

Subjects will return for enrolment between 1 and 3 days prior to challenge. A brief history will be repeated, as well as physical examination, vital signs and sampling for some laboratory evaluations (including TBS/qPCR) ~~and exploratory analyses~~. Participants found to be parasitemic (by TBS and/or qPCR, any parasite density, whether symptomatic or not), will be treated as per Malian guidelines and will be excluded.

Subjects who continue to meet the eligibility criteria will be enrolled and allocated to be inoculated with Pf-infected erythrocytes or PfSPZ Challenge.

Subjects enrolled will be supplied with thermometers, insecticide treated bed nets and insect repellant for use if needed during the course of the study.

8.3. Cohort allocation

This is a non-randomized, open-label study. The 42 participants will be allocated to one of the (sub)cohorts in a pragmatic fashion, generally in order of enrolment. Note that for logistical reasons Cohorts 1, 2 and 3 will not necessarily be conducted in order or in parallel, thus is it may be that one Cohort proceeds with dose-escalation before another Cohort has started.

8.4. Controlled Human Malaria Infection

Subjects that meet the eligibility criteria and are parasite negative by PCR at enrolment will undergo a malaria challenge infection on day 0. Samples will also be collected on the day of challenge (D0) for baseline assessments for some laboratory evaluations (including TBS/qPCR), immunology and other exploratory endpoints. Malaria challenge infection may take place at Sotuba or an associated facility with appropriate infrastructure.

Cohorts 1+2, on the challenge day all subjects will receive a blood-stage inoculum containing 3D7 *P. falciparum* parasites (original QIMR bank or new QIMR bank). Subjects will be cannulated with an indwelling intravenous cannula for the malaria inoculum, and recorded which arm is utilized.
Cohort 3, on the challenge day all subjects will receive a PfSPZ Challenge inoculum by direct venous inoculation. If DVI administration is considered problematic, administration via an indwelling intravenous cannula may also be performed. Sanaria SOPs will be adhered to regarding inoculum preparation and administration.

Supervision by a clinical investigator will be available on-site throughout these procedures and as long as participants are present. Another clinical investigator will be on call, in case of emergency. Emergency aid kits will be present and readily available at all locations, whenever there are participants present. Participants will be observed for a minimum of 60 minutes after administration of the inoculum to evaluate for immediate adverse reactions.

After malaria challenge infection all subjects will be observed closely according to an intensive out-patient follow-up schedule including frequent safety analyses. The study design is illustrated in more detail in the study flowchart. Subjects are required to reside in appropriate accommodation in close proximity to the trial centre. Appropriate accommodation will be determined by the study staff and may include home visits to confirm appropriateness. Participants must sleep under an insecticide treated bed net and must apply insect repellant as directed until the end of study treatment (except during direct skin feeding assays). For all subjects, during this period all relevant investigations will be carried out on an outpatient basis, including frequent safety analyses.

From day 5~~4 (blood-stage challenge) or 6 (sporozoite challenge)~~ assessments of parasite densities using TBS and qPCR will be performed on samples collected daily. Frequency of parasite assessments will be reduced to ~3x per week if (i) the participant remains parasite negative by TBS 21 days following ~~SPZ challenge or 19 days following blood-stage~~ challenge, or (ii) the participant remains parasite negative by TBS for 7 days following piperaquine treatment 1 (T1) or T2. When a TBS or qPCR result is deemed positive for malaria parasites, the technician will inform the trial clinician. All clinical decisions will be based on parasitemia measurements by microscopy and normally qPCR analysis of samples will take place retrospectively. For any individuals who remain negative by PCR until 2~~6 days after blood-stage challenge or until 28 days after sporozoite~~8 days after challenge, end of study treatment will be given, and end of study will apply for that participant. From T3+3 until end of study, if applicable, subjects will only be seen in case of symptoms. All subjects will be seen for a final control visit on day 52 after CHMI. Subjects will be advised to measure their temperature twice daily, and contact the clinical investigators when any symptoms, complaints or fever occurs. The study design is illustrated in more detail in the study flowchart.

Treatment 1 (**T1**, piperaquine 480mg) will be initiated using the following criteria:
1. ≥1000 asexual parasites per µL, *OR*
2. Positive TBS for asexual parasites (any parasite density) in combination with one or more symptoms or signs of malaria.

Treatment 2 (**T2**, piperaquine 960mg) will be initiated using the following criteria:
1. Insufficient clinical response (in the investigator's opinion) following T1, *OR*

2. ≥1000 asexual parasites per µL ≥2 days after T1, *OR*
3. Positive TBS (any parasite density) in combination with one or more symptoms or signs of malaria after initial adequate clinical and parasitological response to T1

~~Treatment with piperaquine (T1 and T2) will be given after a fasting period of ≥ 3 hours. Subjects will be required to fast for a further four hours any time after dosing with piperaquine.~~

Treatment 3 (**T3**, artemether/lumefantrine and primaquine) will be initiated using the following criteria:

1. Insufficient clinical response (in the investigator's opinion) following T2, *OR*
2. ≥1000 asexual parasites per µL ≥2 days after T2, *OR*
3. Positive TBS (any parasite density) in combination with one or more symptoms or signs of malaria after initial adequate clinical and parasitological response to T2
4. If subject remains parasite negative by qPCR until day 28~~6~~ after ~~blood stage challenge or day 28 after sporozoite~~ challenge.
5. At request of the participant if he wishes to withdraw from the study prematurely
6. On any safety grounds by decision of the (clinical) investigator or safety monitor
7. Presumptively at day 49 post-challenge if not already initiated earlier.

If treatment has to be initiated based on the above criteria, the trial clinician will contact the participant for immediate treatment. Preferably, the participant should return immediately or at least within 1 hour. If the participant is not reachable by phone, his/her contact person will be called so contact can be made quickly.

During the entire study period subjects will be instructed to call the trial physicians at any time if they experience severe symptoms. The trial clinician can decide to initiate additional diagnostics, clinical observation/monitoring or (symptomatic) treatment at all times.

During treatment, complaints of malaria infection will be treated symptomatically. In addition to specific treatment with piperaquine, symptomatic treatment will be administered at the discretion of the attending physician. During any assessment for the administration of an antimalarial treatment (T1, T2 or EOS treatment), the Malaria Clinical Score will be calculated by investigators (Appendix 1) for the purpose of future safety assessments. Participants will not be admitted to the hospital during this study, unless the study doctors or the safety monitor deems it necessary, or at the request of the participant.

8.4.1. Dose escalation

Dose escalation within each cohort will proceed *unless* any of the following pre-defined efficacy or safety criteria (below) are met in the preceding dose group of that cohort. If any of the safety criteria are met, or at the discretion of the lead investigator for any reason, a~~n SMC~~ DSMB meeting will be convened to review the data and decide if it is safe to continue with dose escalation. This may include adjustment of the dose escalation schedule, including decreasing or increasing (up to maximally 100-fold) the size of the subsequent dose-escalation step(s).

Pre-defined efficacy criteria:

- all subjects develop parasitemia ≥100 p/µL before day 14

Pre-defined safety criteria:

- initial parasitemia ≥100 p/µL in any subject (any sample within 48 hours of first parasite detection post-inoculation)

AND/OR

- occurrence of any related grade 3 AEs or SAEs in any subject

8.4.2. Physical examination

A complete physical examination will include the examination of general appearance, skin, neck, eyes, throat, lungs, heart, abdomen, back and extremities, and a routine vascular and neurological examination. Height (cm) and body weight (to the nearest ~~0.~~1 kilogram [kg] in indoor clothing, but without shoes) will also be measured, at screening only. Body mass index (BMI) will be calculated using the following formula: BMI = Body weight (kg) / [Height (m)]^2 and converted to an integer.

8.4.3. Vital signs

Vital signs including body temperature, blood pressure (BP) and pulse measurements will be determined and recorded at set time points during the study. Systolic and diastolic BP will be measured while the subject is sitting, using a validated device, with an appropriately sized cuff. In case the cuff sizes available are not large enough for the subject's arm circumference, a sphygmomanometer with an appropriately sized cuff may be used. If vital signs are out-of-range at screening or inclusion, the investigator may obtain two additional readings, so that a total of up to three consecutive assessments are made, with the subject seated quietly for approximately five minutes preceding each repeat assessment. At least the last reading must be within the normal range in order for the subject to qualify.
Temperature will be measured according to local practice, consistently throughout the study. The thermometer used should have a precision of 0.1ºC. The same route should be used throughout the study.

8.4.4. Electrocardiogram

A standard 12 lead ECG will be performed at screening and 4-12 hours after treatment with piperaquine (to coincide with expected maximal piperaquine concentrations after oral dosing). Additional ECG assessments may be performed at any time throughout the study at the discretion of the investigator.
All assessments will occur after the subject has rested for approximately 10 minutes in the supine position. Calibration should be performed per the local site requirements. Each ECG tracing will be labeled with the subject number and date and kept in the source documents at the study site. Interpretation of the tracing must be made by a qualified physician and documented in the Case Report Form (CRF). Minimally, the CRF will contain date and time of ECG, heart rate, PR interval, QRS duration and QT interval (corrected). Clinically significant abnormalities will also be recorded in the CRF and reported to the Safety Monitor.

8.4.5. Blood sampling and safety laboratory evaluations

During the study, blood samples will be drawn for screening, safety and research purposes. The blood sampling schedule in the flowchart shows when blood will be drawn. Hemoglobin, hematocrit, red blood cell count, white blood cell count with differential and platelet count will be measured at regular time points during the study. Creatinine and ALT will be measured at regular time points during the study. ~~Glucose will be measured only at screening.~~ Participants will be tested for RBC alloantibodies before inoculation ~~at screening~~ and at the end of the study.

In the case where a laboratory assessment is outside the reference range for the laboratory at screening and/or inclusion, a repeat measurement may be performed. If still abnormal, a decision regarding whether the result is of clinical significance or not shall be made by the clinical investigator and shall be based, in part, upon the nature and degree of the observed abnormality.
In all cases, the investigator must document in the source documents, the clinical considerations (i.e., result was/was not clinically significant and/or medically relevant) in allowing or disallowing the subject to continue in the study. Blood sampling will be conducted per protocol, although sample volume and tube type may vary pending availability. At the discretion of the PI an equivalent tube or volume may be used for some samples.

8.4.6. Analysis of asexual parasite densities after challenge infection

qPCR for assessment of parasite densities will be performed directly in participant samples, as mentioned previously. qPCR is performed according to a standard procedure as previously described [87] with small adjustments. In short, qPCR will be performed on the multicopy 18S ribosomal RNA gene. All samples are spiked with the extraction control Phocine Herpes Virus (PhHV) to determine efficacy of DNA isolation. Thick smears will be performed directly in participant samples, as mentioned previously and if deemed necessary by the clinical investigator, according to a standard operating procedure which is based on an internationally harmonized protocol for thick smears in CHMIs [36]. Per slide, the number of fields correlating to 0.5 µl of blood will be read. Slides are considered positive if they contain at least 2 unambiguous *P. falciparum* parasites per µl identified and confirmed by a second microscopist in these fields.

8.4.7. Direct Membrane Feeding Assays (DMFA) and Direct Skin Feeding Assays (DFA)

A reproducible *CHMI-transmission* model requires sufficient densities of circulating mature gametocytes in subjects to ensure the generation of adequate numbers of infected *Anopheles* mosquitoes after blood feeding, either directly on the skin (DFA) or through a membrane-covered device that contains a venous blood sample with gametocytes (DMFA).
Following treatment with piperaquine, mosquito feeding assays will be undertaken when gametocytemia appears. To evaluate infectivity in vector mosquitoes we will use a direct membrane feeding assay (DMFA) and a direct skin feeding assay (DFA). This study will perform feeding assays in accordance with MRTC SOPs. These use approximately 100 female mosquitoes for DMFA, and 2 cups of approximately 30 female mosquitoes per cup for the DFA, per time-point to maximize the precision of mosquito infection estimates at low infection rates. For the DMFA, blood will be collected (see flowchart) from each participant for membrane feeding assays with *An. gambiae* mosquitoes. For the DMFA, blood will be kept at 37˚C (to prevent premature exflagellation) for up to 35 minutes until dispensed into membrane feeders. Female mosquitoes (3-6 days old) will be distributed into containers with gauze lids (~100 females/container/time point) and starved overnight prior to feeding on *P. falciparum*-infected blood samples. Mosquitoes will be allowed to feed on the blood through parafilm membranes attached to water jacketed glass feeders attached to a 37°C water bath. Mosquitoes will be allowed to feed for up to 30 min in the dark. Non-engorged mosquitoes will be identified and discarded. After blood feeding, mosquitoes will be maintained in a controlled environment at 26°C, 70-80% RH and provided with 5% glucose as described [88, 89].
For the DFA, 2 cups of approximately 30 mosquitoes will be allowed to bite on the bare skin of the forearm or calf to directly feed for approximately 15 minutes to enable mosquitoes to fully engorge. Participants will be required to wash skin and abstain from insect repellant use or use of other odorized agents as directed in the 24 hours prior to DFA. The experimental infection of mosquitoes by DMFA and DFA will be performed up to 3 times prior to curative anti-malarial treatment at the End of Study.

Seven to 10 days after blood feeding, mosquitoes will be dissected and examined for oocysts in midgut preparations stained with mercurochrome. Oocysts will be counted per mosquito dissected and recorded. Relationship between parasitemia, gametocytemia and mosquito infection (both oocyst prevalence and intensity) will be determined using generalized-linear mixed models [90]. The number of mosquitoes dying prior to dissection will be recorded. For mosquitoes that cannot be examined by microscopy due to unforseen reasons, as a fallback measure, mosquitoes will be stored on day 10 following the blood meal and infection status will be determined by circumsporozoite ELISA, followed by PCR confirmation based on the 18S rRNA target [91, 92].

8.4.8. Quantification of gametocytes, and gametocyte sex ratio

A detailed quantification of gametocytes, gametocyte sex ratio, and gametocyte infectivity will be assessed by using molecular markers, and ex vivo assessments of gametocyte fitness after CHMI

challenge infection. Quantification of gametocytes will be performed using quantitative reverse transcription (qRT)-PCR and a range of molecular markers including but not limited to Pfs25 or CCP4 (female gametocytes), PfMGET (male gametocytes) [51, 93] PfGEXP5 [94], PF14_0748 (young gametocytes) [95] and PF14_0367 (mature gametocytes) [95]. Additional markers of gametocyte maturity and sex ratio will be incorporated as these become available. qRT-PCR determination of circulating gametocytes will be performed retrospectively on at least 3 samples per week.
Blood samples will be taken from EDTA-vials taken for routine qPCR analysis (see flowchart). 100µL will be directly transferred into a cryovial containing RNA preservative for qPCR stage composition analysis. Furthermore, thick smears will be analysed on DMFA time-points to look for gametocytes (Flowchart Study Design).

8.4.9. Immunological assays

Blood samples will be taken for isolation of peripheral blood mononuclear cells (PBMCs) and serum/plasma (see flowchart). PBMCs and sera/plasma will be frozen and can then be used by the MRTC, NIH, Radboudumc or its collaborators for exploratory immunological assays to further analyse the phenotype or functionality of the immune response during and after malaria infection. In order to assess (antigen specific) T cell responses, the HLA-type of participants may be determined. Antibody responses will also be measured to gametocyte antigens, including recombinant proteins Pfs48/45 and Pfs230 using standard ELISA methodologies [96].

8.4.10. Case report forms and data collection

All data collected by the investigators is registered in electronic case report forms. The investigator's notes are collected in subject study files and are considered source data. Since all subjects will be healthy, there is no medical file for the study subjects, with exception of the medical file in case of adverse events/reactions resulting in a medical consultation or hospitalization. In this case the medical file will also be considered as the source data. They will be kept as source documents together with the investigator's notes.

8.4.11. Flowchart Study Design

-	Screening	Enrolment	Challenge	CHMI	-	-	EOS
Visit Number	V1	V3	V4	V5-49			V50
Trial timeline	-120 to -2	-3 to -1	0	4 or 6 onwards[12]	T1	T2	T3	51
Number of visits	1x	1x	1x	1x/day	up to 2x/day	up to 2x/day	1x	1x
Informed consent	X							
Study Comprehension Exam	X							
Eligibility criteria	X	X						
Demographic data, Medical history	X	X						
Physical examination and vital signs	X	X		X[13]	X[13]	X[13]	X[13]	X[13]
Clinical Score (Appendix 1)					X	X	X	
ECG	X				X[14]	X[14]		
Temperature	X	X		X	X	X	X	X
Challenge[1]			X					
Collecting (serious) adverse events	as necessary[11]							
Piperaquine 480mg[2]					X			
Piperaquine 960mg[2]						X		
End of Study treatment (A/L and low-dose primaquine)							X	
Safety report[9]				X				X
Hematology tests[3] (EDTA, 2.0ml)	X	X		X[15]	X[15]	X[15]	X[15]	X[15]
Biochemistry tests[4] (SST, 3.0ml)	X	X		X[15]	X[15]	X[15]	X[15]	X[15]
Serology (SST, 5.0ml)[5]	X	X						
Parasitology[6] (qPCR, EDTA, 0.5ml)	X	X	X	X	X	X	X	X
Parasitology (thick blood smear, EDTA, 0.5ml)	X	X	X	X	X	X	X	X
Parasitology[7] (qRT-PCR, EDTA, 0.5ml)			X	X	X	X	X	X
DMFA and DFA[8] (DMFA: NaHep, 3.0ml)				X				
Exploratory immunology (cellular assays, NaHep, 20ml)		X		X[16]	X		X	X
Exploratory immunology (humoral assays, SST, 5.0ml)		X		X[16]	X		X	X
Exploratory immunology (transcriptional assays, PAXgene/nucleic acid stabilizer, 1.0ml)		X		X[16]	X		X	X
Alloantibody test[17]		X						X
Estimated blood volume collect at visit[10]	11	43						Total: 320

[1] Challenge with blood-stage parasites or sporozoites (Pf SPZ challenge) – see Methods in section 8.4

[2] See treatment criteria section 8.4 Methods

[3] Hemoglobin, hematocrit, platelets, red blood cell count, white blood cell count + differentiation

[4] Creatinine, ALT, LDH. Additional at screening: glucose

[5] HIV, HBV, HCV, *P. falciparum* (screening only)

[6] 18S qPCR for blood stage *P. falciparum*.

[7] Pfs25 qRT-PCR for sexual stage *P. falciparum*.

[8] DMFA and DFAs performed on up to 3 occasions determined by presence of gametocytemia by microscopy or qRT-PCR

[9] A safety report will be compiled upon completion of day 14 for each cohort and at the end of the study for all cohorts.

[10] Total blood volume: ≤500ml. Estimated total blood volume calculated for all routine protocol collections per protocol.
[11] AEs collected from inoculation onwards. (SAE's occurring between enrolment and inoculation will also be recorded).
[12] Follow up begins on day 4 for blood stage inoculation and day 6 for PfSPZ challenge
[13] On indication. Performed at treatment visit only when participants are qPCR positive.
[14] Within 4-12 hours after treatment with piperaquine
[15] Hematology : 2x/wk until T1, T1, T1+2, T2, T2+2, T3 (only if symptomatic), EOS ; Biochemistry : T1, T1+7 (active monitoring); T2, T3 (only if subject symptomatic), EOS. Additional measurements at discretion of investigator.
[16] Exploratory immunology collections at enrolment, day 8 for blood stage inoculation and day 10 for PfSPZ challenge, at T1 (pre treatment), at T3 (pre treatment), and at EOS.
[17] Subjects will be tested for RBC alloantibodies at enrolment and at the end of the study

	Screening	Enrolment	Challenge	CHMI				EOS
Visit Number	V1	V3	V4	V5-49				V50
Trial timeline	-120 to -2	-3 to -1	0	5 onwards[12]	T1	T2	T3	52
Number of visits	1x	1x	1x	1x/day	up to 2x/day	up to 2x/day	1x	1x
Informed consent	X							
Study Comprehension Exam	X							
Eligibility criteria	X	X						
Demographic data, Medical history	X	X						
Physical examination and vital signs	X	X		X[13]	X[13]	X[13]	X[13]	X[13]
Clinical Score (Appendix 1)					X	X	X	
ECG	X				X[14]	X[14]		
Temperature	X	X		X	X	X	X	X
Challenge[1]			X					
Collecting (serious) adverse events	as necessary[11]							
Piperaquine 480mg[2]					X			
Piperaquine 960mg[2]						X		
End of Study treatment (A/L and low-dose primaquine)							X	
Safety report[9]				X				X
Hematology tests[3] (EDTA, 2.0ml)	X			X[15]	X[15]	X[15]	X[15]	X[15]
Biochemistry tests[4] (SST, 3.0ml)	X			X[15]	X[15]	X[15]	X[15]	X[15]
Serology (SST, 5.0ml)[5]	X							
Parasitology[6] (EDTA, 1.0ml)								
Parasitology (18S qPCR)	X	X	X	X	X	X	X	X
Parasitology[7] (gametocyte qRT-PCR)			X	X	X	X	X	X
Parasitology (thick blood smear)	X	X	X	X	X	X	X	X
DMFA and DFA[8] (DMFA: LiHep, 3.0ml)				X				
Exploratory immunology (cellular assays, NaHep, 20ml)			X	X[16]	X		X	X
Exploratory immunology (transcriptional assays, PAXgene/nucleic acid stabilizer, 1.0ml)			X	X[16]	X		X	X
Alloantibody test (EDTA, 6.0mL)[17]			X					X
Estimated blood volume collect at visit[10]	11	1	31					**Total: 230**

[1] Challenge with blood-stage parasites or sporozoites (Pf SPZ challenge) – see Methods in section 8.4
[2] See treatment criteria section 8.4 Methods
[3] Hemoglobin, hematocrit, platelets, red blood cell count, white blood cell count + differentiation
[4] Creatinine, ALT
[5] HIV, HBV, HCV, *P. falciparum* (screening only)
[6] A 1.0ml EDTA will be collected at any visit requesting parasitology PCR and microscopy testing
[7] Pfs25 qRT-PCR for sexual stage *P. falciparum.*

[8] DMFA and DFAs performed on up to 3 occasions determined by presence of gametocytemia by microscopy or qRT-PCR
[9] A safety report will be compiled upon completion of day 14 for each cohort and at the end of the study for all cohorts.
[10] Total blood volume: ≤500ml. Estimated total blood volume calculated for all routine protocol collections per protocol.
[11] AEs collected from inoculation onwards. (SAE's occurring between enrolment and inoculation will also be recorded).
[12] Follow up begins on day 5 following inoculation
[13] On indication. Performed at treatment visit only when participants are qPCR positive.
[14] Within 4-12 hours after treatment with piperaquine
[15] Hematology and biochemistry approximately once per week until T1, at T1, T1+7, T2, T3 and EOS. Additional measurements at discretion of investigator.
[16] Exploratory immunology collections at enrollment, day 9, at T1 (pre-treatment), at T3 (pre-treatment), and at EOS.
[17] Subjects will be tested for RBC alloantibodies at enrollment and at the end of the study

8.5. Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so, without any penalty or loss of medical benefits.

The investigator can decide to withdraw a subject from the study for urgent medical reasons. Participants can be withdrawn from the study procedures at the discretion of the clinical investigator or the local safety monitor for the following reasons:

- Any serious adverse event
- Any adverse event that, according to clinical judgment of the investigator, is considered as a definite contraindication to proceeding with the study procedures.
- The use of concomitant, chronic medication active on the immune system (e.g. steroids, immunosuppressive agents) or with known antimalarial activity against *P. falciparum*
- Withdrawal of informed consent by participant
- Completely lost to follow-up
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- If, on balance, the investigator or safety monitor believes that continuation would be detrimental to the subject's well-being
- Participant non-compliance with study requirements.
- Any other protocol deviation that results in a significant risk to the subject's safety

If a subject withdrawal occurs for any reason, the investigator must make every effort to determine the primary reason for a subject's withdrawal from the study and record this information in the study file. However, in accordance with the principles of the current version of the Declaration of Helsinki, a subject does have 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.

If it is felt that inclusion of the study subject's data for analysis is compromised, the subject will be terminated from the study and data will not be included in analysis. This does not preclude the ethical responsibility of the investigators to ensure the safety of the subject and ensure they receive curative therapy for malaria. If no such compromise is suspected, all data generated before withdrawal will be included in final study analysis. Blood samples collected before withdrawal will be used/stored unless the subject specifically requests otherwise.

For subjects who are lost to follow-up (i.e. those subjects whose status is unclear because they fail to appear for study visits without stating an intention to withdraw), extensive effort (i.e. documented phone calls and e-mails) will be undertaken to locate or recall him or at least to determine his health status. The investigator should show "due diligence" by documenting in the source documents steps taken to contact the subject, e.g. dates of telephone calls, registered letters, home visits etc.

8.6. Replacement of individual subjects after withdrawal

If a subject withdraws prior to inoculation he/she will be replaced with an alternate participant who passed screening, if possible.

8.7. Follow-up of subjects withdrawn from treatment

In the event that a participant discontinues the study for any reason, he/she will be required to complete all safety follow-up as appropriate, as determined by the principal investigator. All participants who have been exposed to CHMI are required to take a curative antimalarial regimen (end of study treatment or alternative effective anti-malarial treatment at the discretion of the clinical investigator).

8.8. Premature termination of the study

The study may be discontinued by the sponsor:

- On advice of the safety monitor
- On advice of the ~~Safety Monitoring Committee (SMC~~Data and Safety Monitoring Board (DSMB~~)~~
- On advice of the clinical investigator
- On advice of the research ethics committee

The safety monitor, ~~SMC~~DSMB, research ethics committee, or investigators may decide to put the study on hold based on adverse events, pending discussion with the Sponsor / ~~SMC~~ DSMB / research ethics committee / safety monitor / investigators. Following discussion, it may be decided to terminate the study. Safety reporting procedures are described in section 9.

9. SAFETY REPORTING

9.1. Temporary halt for reasons of subject safety

The sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify Mali USTTB ethics committee without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending further review by the USTTB ethics committee, except insofar as suspension would jeopardise the subject's health. The investigator will take care that all subjects are kept informed.

9.2. AEs, SAEs and SUSARs

9.2.1. Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to a trial procedure or the experimental intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his or her staff will be recorded.

Abnormal laboratory findings (e.g. clinical chemistry or hematology) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AEs (or SAEs if they meet the definition). The investigator will exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

If there are any severe complaints not typical for malaria infection, such as chest pain, the participant will be evaluated immediately by a qualified clinician using the appropriate clinical assessments (e.g. ECG or measurement of cardiac enzymes) according to standard hospital care.

9.2.2. Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been, based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor and the safety monitor without undue delay after obtaining knowledge of the events (within 24 hours). All SAEs will be reported to the research ethics committee, within 7 days for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report by the investigator/sponsor. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3. Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;

3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the Summary of Product Characteristics (SPC) or IB.

The sponsor will report expedited all SUSARs to FMPOS ethics committee. The expedited reporting of SUSARs is sufficient as notification to the competent authority. The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

As this is an open label study in which the sponsor, investigator and the ~~SMC~~ ~~DSMB~~SMC are not blinded to treatment allocation, the code would not have to be broken in the case of a SUSAR.

Any SAEs, SUSARs or AEs that suggest the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, or that require a change to the protocol or consent will be reported to the research ethics committee in accordance with the reporting requirements of each.

9.3. Follow-up of (serious) adverse events

9.3.1. Adverse event data collection

Safety assessments will be performed and recorded by the investigators. All adverse events/reactions (solicited and unsolicited), noted by the investigators will be accurately documented in the case report form by the investigators. For each event/reaction the following details will be recorded:

1. Description of the event(s)/reaction(s)
2. Date and time of occurrence
3. Duration
4. Intensity
5. Relationship with the intervention
6. Action taken, including treatment
7. Outcome

In addition, symptoms will be ranked as (1) mild, (2) moderate, or (3) severe, depending on their intensity according to the following scale:

- Mild (grade 1): awareness of symptoms that are easily tolerated and do not interfere with usual daily activity
- Moderate (grade 2): discomfort that interferes with or limits usual daily activity
- Severe (grade 3): disabling, with subsequent inability to perform usual daily activity, resulting in absence or required bed rest

If an AE changes in intensity during the specified reporting period, that AE will be updated accordingly.

When an AE/SAE occurs, it is the responsibility of the investigators to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) related to the event. The investigators will then record all relevant information regarding an AE/SAE on the CRF or SAE Report Form, respectively. The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

9.3.2. Assessment of causality

The investigators are obligated to assess the relationship between study procedures and the occurrence of each AE/SAE. The investigators will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant

therapy, other risk factors and the temporal relationship of the event to the challenge will be considered and investigated. The relationship of the adverse event with the study procedures will be categorized as:

Definite	An adverse event for which the study procedure is evidently the cause and for which no other etiology is known to exist
Probable	An adverse event that follows a reasonable temporal sequence from the study procedure and cannot be reasonably explained by the known characteristics of the subject's clinical state.
Possible	An adverse event for which insufficient information exists to exclude that the event is related to the study procedure.
Unlikely	An adverse event which is not reasonably due to study procedures and for which a (much) more likely etiology exists.
Not related	An adverse event for which sufficient information exists to indicate that the aetiology is unrelated either because of the temporal sequence of events or because of the subject's clinical state or other therapies.

Where a binary classification (related/unrelated) is required, definite, probably and possibly related AEs will be classified as related, whereas unlikely and not related adverse events will be considered unrelated.

9.3.3. Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. AEs that result in a subject's withdrawal from the study or that are present at the end of the study will be followed up (if the participant consents to this) until a satisfactory resolution or stabilisation occurs, or until a non-study related causality is assigned.

Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

AEs and SAEs will be reported until end of study, defined as the last patient visit.

9.4. Local Safety Monitor (LSM) and Data and Safety Monitoring Board (~~Safety Monitoring Committee (SMC~~DSMB)~~)~~

9.4.1. Local safety monitor

For this study, a local safety monitor will be appointed, and will be involved in the review of severe and serious adverse events and participant safety. He/she is an experienced clinician qualified to evaluate safety data from clinical studies with malaria infections. He/she is independent of the sponsor and the investigators.

9.4.2. ~~Safety Monitoring Committee (SMC~~DSMB~~)~~ Safety Monitoring Committee (SMC)

A~~n independent Safety Monitoring Committee (SMC)~~ independent Safety Monitoring Committee (SMC)~~DSMB~~ will be appointed, including at least 3 individuals. Their main responsibility will be assessing any severe or serious adverse events and, if necessary, halting further study procedures. A safety report including a list of all reported adverse events and any safety laboratory values outside the normal range will be prepared upon 14 days follow-up of all subjects in cohorts 1a, 2a and 3a, prior to continuation with dose-escalation in groups 2b and 3b, as well as upon completion of the study by all subjects.

The advice(s) of the ~~SMC DSMB~~SMC will be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the ~~SMC~~DSMB~~SMC~~, the sponsor will send the advice to the research ethics committee, including a note to substantiate why (part of) the advice of the ~~SMC DSMB~~SMC will not be followed.

9.4.3. Review of safety data by the safety monitor and ~~SMCDSMB~~SMC

A safety report including a list of all reported adverse events and any safety laboratory values outside the normal range will be prepared upon 14 days follow-up of all subjects in cohorts 1a, 2a and 3a, prior to continuation with dose-escalation in groups 2b and 3b, as well as upon all subjects having completed the study.

These reports will be prepared by a clinical investigator and sent to the safety monitor all clinical investigators involved. The safety monitor will review the safety data within 2 workdays and if warranted instruct the site to take appropriate action.

In addition, safety data for all participants will be assessed by the ~~SMC DSMB~~SMC at the end of the study. Responsibilities of the ~~SMC DSMB~~SMC are described in the ~~SMC DSMB~~SMC Charter. The advice(s) of the ~~SMC DSMB~~SMC will primarily be communicated to the sponsor, who *may* share this with the research ethics committee, but *must* do so if the sponsor chooses not to follow this advice. With this notification a statement will be included indicating whether the advice will/will not be followed.

Any safety laboratory values (other than thick blood smear or qPCR) that lead to immediate malaria treatment will be reported to the safety monitor within 24 hours.

9.4.4. Safety stopping rules

The study may be placed on safety hold for the following reasons:

- On advice of the safety monitor
- On advice of the Principal/Clinical investigators
- On advice of the ~~SMCDSMB~~SMC
- On advice of the research ethics committee
- One or more participants experience a SAE that is determined to be related to the study intervention
- Two or more grade 3 adverse events in the same group of subjects, which are unexpected and possibly, probably or definitively related to the study intervention.
- Any clinical cardiac event that does not meet the criteria of SAE

The safety monitor, research ethics committee, or investigators may decide to put the study on hold based on adverse events, pending discussion with the safety monitor, research ethics committee, and investigators. In addition, the PI can always decide based on characteristics, duration and severity of signs/symptoms to treat and stop the trial for individual cases. The PI will identify when stopping rule criteria are met and alert the appropriate parties. The safety monitor will review all available safety data on a pre-defined time point after the challenge period. If the research ethics committee has recommended safety hold, re-initiation of the study will require research ethics committee concurrence. The research ethics committee will be informed of a safety hold by the sponsor. Following discussion, it may be decided to terminate the study.

10. STATISTICAL ANALYSIS

10.1. Primary study parameter(s)

In this trial, we will determine the suitability of inoculum type and dose to induce gametocytemia, defined as gametocyte prevalence by gametocyte qRT-PCR on any moment during follow-up. Based on preliminary data from a study in The Gambia, after selecting individuals based on their low response to serological markers of malaria exposure, we expect >95% individuals will develop asexual parasitemia. In the CHMI study in malaria exposed individuals in The Gambia, 100% (10/10) of those with low sero-reactivity developed asexual parasitemia greater than 5p/µL within 15 days of challenge [7]. Based on data from the CHMI-transmission studies in malaria naïve individuals, we expect that 100% of those who develop asexual parasitemia >5p/µL will develop gametocytemia ~1-2 weeks later [5, 6, 33]. The CHMI-transmission approach is considered unsuitable if <50% of individuals develop mature gametocytes; the lower limit of the confidence interval around the proportion of gametocyte positive individuals should thus be above 50%. For each inoculum we will evaluate the optimal dose in a total of 10 individuals, 9 of whom should thus become gametocyte positive, the lower limit of the 95% confidence interval around this proportion is 55%.

Comparisons between groups are underpowered by conventional frequentist approaches and a Bayesian model will be used to select the optimal inoculum type and dose for future CHMI-transmission studies with evidence for highest gametocyte prevalence and density.
The aim of this Bayesian analysis is not to test for statistical significance of subtle differences in gametocyte density per arm but rather select the inoculum and dose with the highest level of evidence of sufficiently high gametocyte densities to allow the CHMI transmission model to be used for the evaluation of transmission-blocking interventions.

We will also assess the safety if the inoculums. All adverse events for each participant will be tabulated. Adverse events will be analyzed by calculating the proportion of participants in each group who report mild, moderate or severe adverse events. The frequency of signs and symptoms will be compared between groups with the chi-square test. Any clinically important deviations from normal occurring in routine laboratory test results and/or vital signs as determined by the investigator will be listed. Clinical laboratory data (hematology, blood chemistry) which is outside of the normal range will be listed in tables. Isolated laboratory abnormalities will be reported as AEs if they are considered to be clinically significant by the Investigator. Vital signs which are outside of the normal range and clinically significant will also be listed in tables. All adverse events will be listed by participant and will include details of the treatment received prior to onset, onset time, duration, severity and relationship to the study drug.

10.2. Other study parameters

Demographic data will be summarized by descriptive statistics and will include total number of observations (n), mean, standard deviation (SD) and range for continuous variables and number and % with characteristics for dichotomous variables.

In immunological analyses we will assess differences by comparing mean values between groups or time points using either a two-tailed student's t-test (if comparing two groups) or a one-way ANOVA (if comparing more than two groups) or non-parametric equivalents. Paired tests will be used if pre-intervention values are compared with post intervention values, unpaired tests will be used if comparisons are made between groups. For discrete variables (e.g. the number of positive assays), the chi-squared test or Fisher's exact test will be used (two-tailed).

11. ETHICAL CONSIDERATIONS

11.1. Regulation statement

This study will be conducted in accordance with the latest Fortaleza revision of the Declaration of Helsinki (2013), the ICH Good Clinical Practice, and Malian law and regulatory requirements.
The investigators are responsible for obtaining all relevant Research Ethics Committee (EC) / Institutional Review Board (IRB) approvals for the protocol and any subsequent amendments in compliance with local law before the start of the study.

11.2. Recruitment and consent

As soon as the study is approved by the FMPOS ethics committee, healthy participants will be recruited to participate in the study. See also 8.2.1. Community meetings will be organized to explain the study objectives and procedures. After this first step in the sensitization, potential participants are invited for information sessions to explain in more detail study objectives and procedures. Participant information files will be made available in French. This information is read out aloud and given in printed form. Participants are explained throughout the process that: i) participation is entirely voluntary; ii) persons may withdraw from participation in this study or any part of the study at any time without any need to justify their reasons for withdrawal; iii) they should ask any questions and only participate if they fully understand the aim of the study and its consequences. During and after the meeting there will be time for questions. After this free discussion with the investigator, and any follow-up discussion if necessary, the participant will be given sufficient time to consider participation. Participants will be required to complete a study comprehension exam with the investigator to confirm their understanding of the study. A score of >80% is required to for inclusion. A subject may repeat the comprehension exam if required after further discussion with the investigator until a satisfactory score is reached.

Participants who are interested in participating will be asked to fill in the application form and will be invited to come for a screening visit. Eligible subjects may only be included in the study after providing written, FMPOS-approved informed consent. If a subject is unable to read, an impartial literate witness will be present to verify informed consent of the participant and will counter-sign the informed consent form. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol, including screening procedures). The process of obtaining informed consent should be documented in the subject source documents. During the screening visit, the study comprehension exam answers will be discussed and inclusion and exclusion criteria will be checked. Again, the investigator will answer any questions the participant has. The possibility of withdrawal from the study, at any time, without penalty and without any declaration of the reason will be pointed out to the participants.

The investigators will be responsible for providing adequate verbal and written information regarding the objectives and procedures of the study, the potential risks involved and the obligations of the participants. Participants will be informed that they will not gain health benefits from this study. Trainees or other students who might be dependent on the investigators or the study group will not be included in the study.

11.3. Benefits and risks assessment, group relatedness

Two major areas of ethical concern are contained within this proposal, namely the use of blood from humans and the use of human participants for *P. falciparum* CHMI. All partners in this proposal are aware of and follow the relevant national and international rules and regulations as they pertain to access to material of human origin and clinical research. International agreements such as the Declaration of Helsinki will be observed and respected.

11.4. Ethical aspects concerning the use of human participants

Infection of humans with malaria has been carried out for nearly a century, including for therapeutic use as treatment for neurosyphilis and later for drug and vaccine evaluation. The ability to carry out this type of work is largely based on the relatively low morbidity and the lack of mortality seen in these studies since the advent of feeding mosquitoes on *P. falciparum* gametocyte cultures in 1986 [39]. Testing in human subjects remains the only reliable and convincing way to obtain information on the immunological responses that are important for protection against malaria. Of course, the compelling need for a malaria vaccines and treatments needs to be balanced with the potential risks and discomforts of the participants. Explorative studies looking for new or complementary transmission blocking drugs or vaccines are of paramount importance with the potential of large-scale application in endemic countries. These transmission-blocking interventions are considered essential tools to consolidate recent gains of malaria control in recent years and move towards malaria elimination.

The study will be undertaken in accordance with GCP, according to the standards defined in the EEC directive 91/507/EEC, and in the Directive on Good Clinical Practice in Clinical Trials (ICH GCP, 75/318/EEC, January 1997) and under the principles of the Declaration of Helsinki; ethical permission will be sought from FMPOS Ethics Committee.

11.5. Compensation for injury

The sponsor/investigator has a liability insurance which provides cover for damage to research subjects through injury or death caused by the study.

11.6. Incentives

Enrolled and challenged participants will receive compensation for their time and for the inconveniences of taking part in this study. This is based on compensation fees for procedures as below:

- Inconvenience of blood tests and/or visits: USD $6 (or equivalent) per scheduled visit with blood sampling or USD $3 per visit without blood drawn
- Malaria challenge: USD 6$ per participant
- Illness and treatments compensation: USD $6 for unscheduled visits requiring blood collection
- Direct Feeding Assays: USD $6 per DFA

Travel expenses will not be additionally reimbursed, and compensation will not be provided to participants who are not enrolled i.e. screen failures. Eligible participants who are enrolled at the inclusion visit as back-ups, but who are not challenged on Day 0 will be compensated USD $6 per participant. These compensation amounts are reasonable and in line with Malian common practice. In case of unexpected medical complications, there will be access to state-of-the-art medical treatment with full costs covered by the insurance of MRTC.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1. Handling and storage of data and documents

12.1.1. Confidentiality

All parties agree to adhere to the principles of medical confidentiality in relation to clinical study subjects involved in this trial and shall not disclose the identity of subjects to third parties without prior written consent of the subject.

Subjects will not be identified in any publicly released reports of this study. All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator. The investigator will inform the subjects that the above-named representatives will review their study-related records without violating the confidentiality of the subjects. All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number in order to maintain subject confidentiality. All records will be kept locked and all computer entry and networking programs will be done with coded numbers only.

The investigator will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH GCP Guidelines, any regulatory requirements, and institutional requirements for the length of storage and for the protection of confidentiality of participants. The investigator will permit direct access to study records and source documents to authorized representatives of the sponsor, ethical committee(s)/institutional review board(s), regulatory agencies, authorised individuals from Radboudumc, NIAID/NIH, Sanaria and QIMR, and the external monitor(s), for the purposes of quality assurance reviews, audits / inspections, and evaluation of the study safety and progress. Direct access includes examination, analysis, verification, and reproduction of de-identified records and reports that are important to the evaluation of the trial. Data and biological samples will be stored for 15 years. Biological samples from this study will be stored for 15 years for research related to malaria immunology and transmission dynamics. New immunological or molecular tests may become available in the future that could strengthen or validate this research or help find important new findings. Samples will only be used for malaria-related research.

12.1.2. Data collection

Designated trial staff will enter the data required by the protocol the CRF.

Data from CRFs will be collected directly from subjects during study visits and telephone calls. CRFs will be used as source. Clinical data will be entered directly into the electronic CRF (eCRF) study-specific DataFax electronic database. Where direct entry is not possible, paper CRFs wil be used before manually entering into the electronic database. Any type of corrections to paper CRFs must be initialed and dated by the person making the correction. All CRFs should be reviewed by the Investigator and signed as required with written signature.

Data entry will be performed by authorized individuals. Corrections to the electronic data systems will be tracked electronically (password protected or through an audit trail) with time, date, individual making the correction, and what was changed. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

12.1.3. Database management and quality control

All data should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification. An external monitor will review the data entered into the eCRFs by investigational staff for completeness and accuracy and will instruct the site personnel to make any

required corrections or additions. Queries are made during each monitoring visit. Designated investigator site staff are required to respond to the queries and confirm or correct the data. Medical history/current medical conditions and adverse events will be coded using MedDRA terminology.

12.2. Monitoring and Quality Assurance

Before study initiation, the protocol and eCRFs together with relevant SOPs will be reviewed by the sponsor, the investigators and their staff. During and after completion of the study, the data monitor will visit the site to check the completeness of records, the accuracy of entries on the eCRFs, the adherence to the protocol and to Good Clinical Practice, the progress of enrolment, and to ensure that piperaquine, A/L and primaquine are being dispensed and accounted for according to protocol. The investigator will maintain source documents for each subject in the study, consisting of case and visit notes containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments. All information on eCRFs must be traceable to these source documents in the subject's file. As with all parts of the eCRF, there is an audit trail in place to register every data entry. The investigator will also keep the original informed consent form signed by the subject (a signed copy is given to the subject).

The investigator will give the external monitor access to all relevant source documents to confirm their consistency with the eCRF entries. The monitor will perform full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria and documentation of SAEs. The recording of data that will be used for all primary and safety variables will be assessed for at least 25% of included subjects.

12.3. Amendments

Amendments are changes made to the research and will only be made after favourable opinions / approvals by the FMPOS Ethics Committee have been given - except where necessary to eliminate apparent immediate hazards to the subject(s). All amendments will be submitted to the FMPOS Ethics Committee for review and approval.

12.4. Public disclosure and publication policy

The final report will be prepared by the investigators. It will be signed by the project leader or the principal investigator. The investigators will make every effort to publish the results in a peer-reviewed journal. Depending on journal requirements, data may be deposited in an online repository for review and secondary analyses. Participants provide explicit consent for this procedure.

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13. APPENDICES

13.1. Appendix 1

Clinical Score for Malaria

Clinical Score	
Symptom	**Clinical Score** *0 - Absent, 1 - Mild, 2 - Moderate, 3 – Severe*
Headache	
Myalgia (muscle ache)	
Arthralgia (joint ache)	
Fatigue/lethargy	
Malaise (general discomfort/uneasiness)	
Chills/shivering/rigors	
Sweating/hot spells	
Anorexia	
Nausea	
Vomiting	
Abdominal discomfort	
Fever	
TOTAL SCORE	

Recorder's signature: _____ Date: _____

13.2. Appendix 2

Protocol: Pf CHMI-trans
Subject ID:
Date:

Study Comprehension Exam

	True	False
1. I can take part in other studies of investigational vaccines or investigational drugs while in this study	☐	☐
2. The malaria parasites injection I will receive are dead malaria parasites	☐	☐
3. The main purpose of the study is carefully grow malaria parasites in participants and see if they can be transmitted to laboratory mosquitos	☐	☐
4. I can travel out of town and still take part in the study as long as I tell the investigators	☐	☐
5. I need to minimize my risk of being bitten by mosquitoes during the study by sleeping under a bed net every night and using repellant as directed	☐	☐
6. One of the study risks is having side effects from the antimalarial drugs I am given	☐	☐
7. I may get severe malaria if I do not fully comply with the study drugs given to me	☐	☐
8. Participation in this study will last about two months	☐	☐
9. If I get symptoms of malaria, I will be given treatment by study staff	☐	☐
10. I can take treatments at home if I have them without talking to study staff	☐	☐
11. I will have blood drawn at most study visits	☐	☐
12. Some of my blood will be stored in a research lab and may be used for future research	☐	☐
13. I will be tested for HIV, hepatitis B and hepatitis C prior to enrolling into the study.	☐	☐
14. It is not important if I skip 1 or 2 study appointments	☐	☐
15. I may be removed from the study by the investigators if they feel it is unsafe for me to continue	☐	☐
16. Study staff may visit my accommodation to make sure it safe and I am following study directions	☐	☐
17. I may develop symptoms of malaria infection (fever, chills, nausea, vomiting, muscle aches, joint pains) during my participation in this study	☐	☐
18. If I have a problem at night I must wait until the morning to tell study staff	☐	☐
19. Participation in this study is completely voluntary	☐	☐
20. In order to participate in this study, I will have to provide emergency contacts to the study staff and be reachable by phone 24/7.	☐	☐

Correct Answers:_____ Incorrect Answers:_____ Score: / (%)

A score of >80% is required for enrolment

Participant name:_____ Date:_____ Signature:_____

DD/MMM/YYYY

Investigator name:_____ Date:_____ Signature:_____

DD/MMM/YYYY

Witness name:_____ Date:_____ Signature:_____

DD/MMM/YYYY

Study Comprehension Exam (answers)

	True	False
1. I can take part in other studies of investigational vaccines or investigational drugs while in this study	□	X
2. The malaria parasites injection I will receive are dead malaria parasites	□	X
3. The main purpose of the study is carefully grow malaria parasites in participants and see if they can be transmitted to laboratory mosquitos	X	□
4. I can travel out of town and still take part in the study as long as I tell the investigators	□	X
5. I need to minimize my risk of being bitten by mosquitoes during the study by sleeping under a bed net every night and using repellant as directed	X	□
6. One of the study risks is having side effects from the antimalarial drugs I am given	X	□
7. I may get severe malaria if I do not fully comply with the study drugs given to me	X	□
8. Participation in this study will last about two months	X	□
9. If I get symptoms of malaria, I will be given treatment by study staff	X	□
10. I can take treatments at home if I have them without talking to study staff	□	X
11. I will have blood drawn at most study visits	X	□
12. Some of my blood will be stored in a research lab and may be used for future research	X	□
13. I will be tested for HIV, hepatitis B and hepatitis C prior to enrolling into the study.	X	□
14. It is not important if I skip 1 or 2 study appointments	□	X
15. I may be removed from the study by the investigators if they feel it is unsafe for me to continue	X	□
16. Study staff may visit my accommodation to make sure it safe and I am following study directions	X	□
17. I may develop symptoms of malaria infection (fever, chills, nausea, vomiting, muscle aches, joint pains) during my participation in this study	X	□
18. If I have a problem at night I must wait until the morning to tell study staff	□	X
19. Participation in this study is completely voluntary	X	□
20. In order to participate in this study, I will have to provide emergency contacts to the study staff and be reachable by phone 24/7.	X	□