CONFIDENTIAL

CLINICAL TRIAL PROTOCOL

Safety and protective efficacy of genetically modified *Plasmodium berghei (Pb(PfCS@UIS4))* malaria parasites in healthy volunteers

Version 4.0, 28-November-2017

(“*PbVac*”)

PROTOCOL TITLE: 'Safety and protective efficacy of genetically modified *Plasmodium berghei (Pb(PfCS@UIS4))* malaria parasites in healthy volunteers'

Protocol ID	**PbVac**
Short title	Safety and protective efficacy of Pb(PfCS@UIS4)
Toetsingonline number	NL60019.091.16
EudraCT number	2017-000315-17
WIRB protocol number	1172775
Version	**Version 3.0**
Date	**28-02-2017**
Project leader/Clinical investigator	**Isaie J. Reuling, M.D.** Radboudumc, Department of Medical Microbiology Tel: +31 (0)24 3619515 Fax: +31 (0)24 3635153 E-mail: isaie.reuling@radboudumc.nl
Sponsor	Stichting Katholieke Universiteit Radboud University Medical Centre (Radboudumc) Department of Medical Microbiology Geert Grooteplein-Zuid 10 6525 GA, Nijmegen, The Netherlands
Principal Investigator	**Prof. Robert W. Sauerwein, M.D.** Radboudumc, Department of Medical Microbiology Tel: +31 (0)24 3610577 Fax: +31 (0)24 3635153 E-mail: robert.sauerwein@radboudumc.nl
Co-Principal Investigator	**Perry van Genderen, MD PhD** Department of Internal Medicine Havenziekenhuis Tel: +31 (0)10 23443616 Fax: +31 (0)10 4121645 E-mail: p.van.genderen@havenziekenhuis.nl

Clinical investigator	**Gerdie M. de Jong, M.D.** Department of Internal Medicine Havenziekenhuis Tel: +31 (0)615193584 Fax: +31 (0)10 4121645 Email: g.m.dejong@erasmusmc.nl
Diagnostic laboratory investigator	**Dr. Jaap van Hellemond** Dept. Medical Microbiology & Infectious Diseases Erasmus MC & Havenziekenhuis Tel: +31 (0)6 22273138 j.vanhellemond@erasmusmc.nl
Funding partner	**PATH Malaria Vaccine Initiative (MVI)** 2201 Westlake Avenue, Suite 200 Seattle, Washington 98121, USA
Biological evaluator	**Dr. Rob Hermsen, PhD** Radboudumc, Department of Medical Microbiology Tel: +31 (0)24 3613663 E-mail: Rob.Hermsen@radboudumc.nl
Independent expert (s)	**Dr. Els van Nood, MD PhD** Department of Internal medicine & Infectious Diseases Erasmus MC, Rotterdam Tel: +31 (0)6 47104092 Email: e.van.nood@havenziekenhuis.nl
Laboratory sites	Safety parameters: Havenziekenhuis/Erasmus MC Dept. Clinical Chemistry, Hematology and Medical Microbiology & Infectious Diseases Immunology laboratories: **IAVI-Human Immunology Laboratory** Imperial College London, Faculty of Medicine 2nd Floor Lift Bank D

	Chelsea and Westminster Hospital 369 Fulham Road, London, UK SW10 9NH **Malaria Serology Lab** Malaria Vaccine Branch Walter Reed Army Institute of Research Room 3W80 503 Robert Grant Avenue Silver Spring, Maryland 20910-7500 **Instituto de Medicina Molecular – IMM** Faculdade de Medicina da Universidade de Lisboa Miguel Prudêncio Av. Professor Egas Moniz 1649-028 Lisboa Portugal **Radboud University Medical Centre (Radboudumc)** Department of Medical Microbiology Geert Grooteplein-Zuid 10 6525 GA, Nijmegen, The Netherlands
Pharmacy	Pharmacy Lagaay Van Helmontstraat 99, 3029 AA Rotterdam - The Netherlands Pharmaceutical company
External monitor	**CROMSOURCE** Lange Dreef 11H 4131 NJ Vianen, The Netherlands Tel: +31 (0)347 344977 ext. 2614 Fax: +31 (0)347 34 42 89 Mobile: +31 6 13516468

PROTOCOL SIGNATURE SHEET

Name	**Signature**	**Date**
Sponsor and principal investigator: ***Robert Sauerwein, MD PhD*** *Department of Medical Microbiology* *Radboud university medical center*		
Co-Principal investigator: ***Perry van Genderen, MD PhD*** *Department of Internal Medicine* *Havenziekenhuis*		
Project leader: ***Isaie Reuling, MD*** *Department of Medical Microbiology* *Radboud university medical center*		

Version History

Version	**Date**	**Author(s)**	**Summary of changes**
2.0	24 January 2017	Isaie Reuling Robert Sauerwein Perry van Genderen	- Information on LFT derangements in CHMI (section 1.5) - Additional inclusion criterium (section 4.2) - Additional biochemistry test measurements (flowchart) - Adjustment to Investigational and non-investigatonal product (Section 6) - Added information on SUSARs (section - Minor revision in section power size calculation (section 4.4)
3.0	28 February 2017	Isaie Reuling Robert Sauerwein Perry van Genderen	- minor adjustment to section 1.5 LFT derangements. - additional safety/exclusion criteria renal function (section 1.5)
4.0	28 November 2017	Gerdie de Jong Isaie Reuling Robert Sauerwein Perry van Genderen	- Additional exploratory objectives and endpoints (Summary, section 2, section 7.1.2.) -Rationale of sampling on day 4 and 5

			post-CHMI for group 4 and 5 and additional details on the exploratory analysis (section 7.3.13).

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

A.	***Anopheles***
ACT	**Artemisinin-based Combination Treatment**
AE	**Adverse Event**
AL	**artemether–lumefantrine**
ALT	**Alanine Aminotransferase**
ANOVA	**analysis of variance**
BMI	**Body Mass Index**
BP	**Blood Pressure**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CHMI	**Controlled Human Malaria Infection**
CRF	**Case Report Form**
CPS	**chemoprophylaxis and sporozoites**
CSP	**Circumsporozoite protein**
CRO	**Contract Research Organization**
CV	**Curriculum Vitae**
ECG	**ElektroCardioGram**
EDTA	**Ethylenediaminetetraacetic acid**
eCRF	**electronic Case Report Form**
ELISA	**Enzyme-Linked Immuno Sorbent Assay**
G6PD deficiency	**Glucose-6-phophatedehydrogenase deficiency**
GAP	**Genetically Attenuated Parasites**
GCP	**Good Clinical Practice**
GP	**General Practitioner**
HBV	**Hepatitis B Virus**
HCV	**Hepatitis C Virus**
HIV	**Human Immunodeficiency Virus**
HTLV	**HumanT-lymphotropic Virus**
IC	**Informed Consent**
IFN-γ	**Interferon-gamma**

IRB	**Institutional Review Board**
IV	**Intravenous**
LDH	**Lactate dehydrogenase**
LSM	**Local Safety Monitor**
mAbs	**Monoclonal antibodies**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
MFS	**Membrane Feed for Sporozoite production**
NF54	**Nijmegen *falciparum* strain 54**
P.	***Plasmodium***
par/ml or p/ml	**parasites per milliliter**
PATH-MVI	**PATH Malaria Vaccine Initiative**
PATH REC	**PATH Research Ethics Committee; the funding partner's ethical committee.**
Pb(PfCS@UIS4)	**Transgenic Plasmodium berghei parasite expressing P. falciparum CS under the control of the P. berghei UIS4 promoter**
PBMC	**Peripheral Blood Mononuclear Cell**
PCR	**Polymerase Chain Reaction**
Pf	***Plasmodium falciparum***
qPCR	**Real-time Quantitative Polymerase Chain Reaction**
Radboudumc	**Radboud university medical center**
RAS	**Radiation Attenuated Parasite**
SAE	**Serious Adverse Event**
Sanquin	**Sanquin Blood Supply Foundation, who on the basis of the Blood Supply Act is responsible for all blood supply (blood and blood products) for transfusion in The Netherlands.**
SCORE	**Systematic Coronary Risk Evaluation**
SMC	**Safety Monitoring Committee**
SOP	**Standard Operating Procedure**
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**

SWAB	**Stichting Werkgroep Antibioticabeleid**
WIRB	**Western Institutional Review Board; PATH's designated IRB to who PATH REC delegates ethical review to WIRB for vaccine studies.**
WHO	**World Health Organization**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**
ZAVIN	**Ziekenhuis Afval Verwerkings Installatie Nederland (Hospital Waste Disposal company)**
γGT	**Gamma Glutamyl Transferase**

SUMMARY

Rationale:

Malaria is caused by *Plasmodium* (*P.*) parasites and is one of the major infectious diseases in the world, with a tremendous impact on the quality of life, significantly contributing to ongoing poverty in endemic countries. Whole organism malaria vaccine approaches generate high-level (>90%) protection against malaria in humans through i) immunization with sporozoite forms of the parasite attenuated by irradiation or ii) when sporozoites are administered together with a chemoprophylactic dose of chloroquine[1, 2] In the underlying study, a genetically modified *P. berghei* parasite is used. *P. berghei* is one of the four *Plasmodium* species that causes malaria in rodents. The hypothesis is that immunization of humans with *P. berghei* will induce a cross-species immune response without the risk of a breakthrough infection. To further increase the potential for protective efficacy, the *P. falciparum* circumsporozoite (CS)- protein gene has been integrated in the *P. berghei* parasite, generating a genetically modified *P. berghei* parasite, abbreviated as *Pb(PfCS@UIS4)*.

Objectives:

The study is divided in phase 1 and phase 2.

Phase 1

Primary objective:

To determine the safety and tolerability of administration of *Pb(PfCS@UIS4)* to healthy volunteers delivered by infectious mosquito bites.

Secondary objective:

Immunogenicity of Pb(PfCS@UIS4) as assessed by ELISA and IFA.

Exploratory objective:

To analyse immune responses in volunteers immunized by *Pb(PfCS@UIS4)*.

Phase 2

Primary objective:

To determine the safety, tolerability and protective efficacy of immunization with *Pb(PfCS@UIS4)* against Controlled Human Malaria Infection (CHMI) by mosquito bite.

Secondary objective:

Immunogenicity of Pb(PfCS@UIS4) as assessed by ELISA and IFA.

Exploratory objective:

To analyse immune responses in volunteers exposed to *Pb(PfCS@UIS4)* and to determine cytokine responses, coagulation activation and inflammation parameters in non-immune individuals infected with *P. falciparum* during malaria liver phase

Study design:

Multicenter, open label, adaptive design, study

Phase 1

- (Group 1 and 2): dose escalation, safety trial (n=6)
- (Group 3): dose escalation, safety trial (n=12)

Phase 2

- (Group 3): protective efficacy trial
- (Group 4): infectivity control group, challenge 1 (n=6)
- (Group 5): infectivity control group, challenge 2 (n=6)

Study population:

Phase 1: Eighteen healthy male and female volunteers, aged 18 to 35 years, will participate in the study.

Phase 2: The same twelve volunteers of group 3, will subsequently participate in Phase 2 of the study. In addition, six volunteers will be recruited as infectivity control subjects per challenge and will receive 5 infective mosquito bites with NF54-*P. falciparum* parasites.

Intervention:

Phase 1: A total of eighteen healthy adult volunteers will be recruited across 3 groups. Three volunteers (Group 1) will be exposed to bites of five *Pb(PfCS@UIS4)*-infected mosquitoes. Volunteers will be closely monitored for adverse events for a period of 28 days. Upon satisfactory safety assessment of group 1, an additional three volunteers (group 2) will be exposed to 25 *Pb(PfCS@UIS4)*-infected mosquito bites. Upon satisfactory safety assessment of group 2, an additional 12 volunteers (group 3) will be exposed to 75 *Pb(PfCS@UIS4)*-infected mosquito bites. If one of the volunteers is not fit to participate in the study on day 0, an alternate included volunteer will replace him/her. For this purpose one additional volunteer will be screened for possible back up in groups 1 and 2, and two additional volunteers will be screened for possible back up for group 3.

All exposed volunteers are subjected to close follow-up after exposure at the Havenziekenhuis, with regular visits to the clinical trial centre, periodic physical examinations, frequent blood sampling, and recording of adverse events in a diary (see section 7.3. All exposed volunteers will be treated with a curative regimen of Malarone (atovaquone/proguanil), either at the time of detection of blood stage parasitemia by thick smear, or 28 days after exposure to *Pb(PfCS@UIS4)*-infected mosquitoes for groups 1 and 2. Volunteers of group 3 will subsequently enter phase 2, if phase 1 is considered safe, and if no blood stage parasitemia is detected during follow up. End of follow-up will be 100 days after exposure to *Pb(PfCS@UIS4)* for groups 1 and 2, and group 3 in case this group is not proceeding to phase 2.

Phase 2: The trial objective is to evaluate the safety and protective efficacy of repeated exposure to *Pb(PfCS@UIS4)*-infected mosquito bites by subsequent CHMI of test subjects. The same volunteers of group 3 will be exposed three additional times to the bites of 75 *Pb(PfCS@UIS4)*-infected mosquitoes *(n=12),*at four to eight week intervals (at weeks 4, 4, 4-8). Three to four weeks after the last exposure, all volunteers will undergo a CHMI with five *P. falciparum* (NF54)-infected mosquitoes. Six infectivity control subjects will be recruited to receive 5 *P. falciparum* (NF54) infective mosquito bites per challenge infection.

Based on the first results of phase 2 an *ad hoc* decision will be made to include a potential booster immunization and/or subsequent second challenge infection with five *P. falciparum* (NF54)-infected mosquitoes. The booster immunization and/or subsequent second challenge infection will be initiated using the following criteria:

1. In the event that 2 to 4 volunteers are sterilely protected, all volunteers (protected and unprotected) will receive a booster immunization followed by a second challenge infection 3 months after the booster immunization.
2. In case ≥5 volunteers are sterilely protected, all unprotected volunteers will receive a booster immunization followed by a second challenge infection 3 months later, all protected volunteers will not be boosted and will only receive a second challenge infection to assess durability of protection.

All exposed volunteers are subjected to close follow-up after exposure, with regular visits to the clinical trial centre, periodic physical examinations, frequent blood sampling and adverse events will be recorded in a diary. For all subjects, during this period, all relevant investigations will be carried out on an outpatient basis, including frequent safety analyses.

Exposed volunteers will be treated with a curative regimen of Malarone (atovaquone and proguanil), at the time of detection of blood stage parasitemia by thick smear during immunizations. During the challenge infection, all volunteers will receive a curative treatment, either at the time of blood stage parasitemia by qPCR or day 28 post CHMI. End of follow up will be 100 days after the last exposure to *Pb(PfCS@UIS4)* and 35 days post CHMI for the infectivity control subjects.

Study parameters/endpoints:

Phase 1 (Safety)

Primary endpoints:

- Frequency and magnitude of adverse events in study groups.
- Presence of parasitemia after exposure to *Pb(PfCS@UIS4)*, as assessed by thick smear.

Secondary endpoints:

- Immunogenicity of Pb(PfCS@UIS4) as assessed by ELISA and IFA.

Exploratory endpoints:

- Cellular and humoral immune responses after exposure to *Pb(PfCS@UIS4)*.

Phase 2 (Efficacy)

Primary endpoints:

- Frequency and magnitude of adverse events after multiple exposures to *Pb(PfCS@UIS4).*
- Time to parasitemia after CHMI with the wild-type NF54 *P. falciparum* strain, as detected by qPCR.

Secondary endpoints:

- Immunogenicity of Pb(PfCS@UIS4) as assessed by ELISA and IFA.

Exploratory endpoints:

- Cellular and humoral immune responses after exposure to *Pb(PfCS@UIS4)* and challenge with *P. falciparum*.
- Cytokines response, coagulation activation and inflammation parameters after challenge with *P. falciparum.*

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

Benefits: There are no direct personal benefits for volunteers. Volunteers will be advised to take regular malaria chemoprophylaxis when travelling to malaria endemic areas in the future.

Risks: Risks for volunteers are related to i) potential breakthrough blood stage infection after exposure to *Pb(PfCS@UIS4)* sporozoites, ii) potential side effects of atovaquone/proguanil treatment and iii) side effects related to exposure to multiple mosquito bites. Control volunteers from group 4 (and group 5), and immunized non-protected volunteers in Phase 2 are expected to experience blood stage malaria infection.

Burden:

In Phase 1 volunteers are exposed to 5, 25 or 75 mosquito bites. After each exposure, there will be a short period (28 days) of intense clinical monitoring, with frequent clinical site visits and blood examinations. In Phase 2, volunteers are exposed to 75 mosquito bites on 3 occasions followed by an additional 5 mosquito bites for the NF54 *P. falciparum* challenge infection. After the challenge infection there is a short period (28 days) of intense clinical monitoring with frequent site visits and blood examinations. Depending on the results of phase 2 a booster immunization and/or second challenge infection is initiated. It is expected that volunteers will have to visit the trial centre on 20-25 occasions for Phase 1 and 36 for Phase 2. If the extra booster and/or second challenge is initiated, volunteers will have 23-27 more visits. In Phase 1, the maximum cumulative amount of blood collected will be 244 or 321 ml for each volunteer, depending on the proceeding to phase 2. In Phase 2, the maximum amount of blood collected per volunteer will be 186 ml during the immunization and 521 ml during the challenge phase. In case a booster and/or subsequent challenge infection is initiated, a maximum of 508 ml of blood will be collected. In total, not more than 1.5 L of blood will be collected per volunteer in the entire study. In addition, physical examinations will be performed when clinically indicated and subjects will be asked to complete a diary.

INTRODUCTION AND RATIONALE

1.1 Introduction

Malaria is a common and serious tropical disease. It is a protozoan infection transmitted to human beings by *Anopheles* mosquitoes. Human malaria is caused by five species of *Plasmodium (P.)* protozoa: *P. falciparum (Pf)*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Malaria is a public health problem in over 90 countries worldwide, inhabited by some 40% of the world's population, i.e. over 3 billion people are at risk of being infected with malaria. It has been estimated that the incidence of malaria in the world is around 214 million clinical cases each year[3]. People living in Sub-Saharan Africa account for 88% of these cases. Malaria mortality is estimated at 438,000 deaths worldwide per year[4]. Most malaria deaths occur among young children in Africa, especially in remote rural areas with poor access to health services. Other high-risk groups include pregnant women, non-immune travellers, refugees, displaced persons, and labour forces entering into endemic areas. The epidemiology of malaria has been changing over recent years due to a combination of factors including increasing resistance of malarial parasites to chemotherapy, increasing insecticide resistance of the *Anopheles* mosquito, ecological and climate changes and increased international travel to malaria-endemic areas.

Parasites (sporozoite stage) are injected into the skin capillaries by a female *Anopheles* mosquito. From there, they travel via the bloodstream to the liver, where they develop and multiply in liver cells before re-entering the blood stream (merozoite stage) and invading erythrocytes for further reproduction. The liver stage of infection is obligatory, but completely asymptomatic. Clinical malaria is caused by the cyclical proliferation of asexual stages in erythrocytes, during the subsequent blood stage of infection. Malaria mortality is primarily due to organ dysfunction, amongst which the brain, following sequestration of infected erythrocytes in the micro-vasculature.

There are several reasons why malaria continues to be one of the greatest health problems in worldwide. One of the main reasons is the difficulty in achieving adequate coverage with, and reducing pricing of, existing tools such as drugs, insecticide-treated bed nets, and, nowadays, counterfeit drugs. The decreasing effectiveness of existing tools (e.g. emergence of anti-malarial resistance by the parasite, resistance to insecticides, including pyrethroids, by the mosquitoes) is a major challenge. The availability of an efficacious malaria vaccine would certainly be a major achievement to overcome the shortcomings of current malaria control strategies.

1.2 Rationale

Both natural and controlled exposure to malaria parasites can lead to the development of protective immunity, providing a foothold for the development of a vaccine[2, 5-8]. The clinical development of a malaria vaccine has been a continuous effort over the past half century[9], following the traditional vaccine development approach. Different formulations of a number of antigens and/or adjuvants have been tested in Phase I trials but only about a dozen candidates have been evaluated in Phase II clinical field trials[10]. No vaccine has worked well and most candidates have failed completely. The most advanced vaccine to date, RTS,S, delays patency and reduces clinical severity, but does not provide long term protection against infection[11-13]. Although a milestone in itself, and potentially an additional tool in the combat against malaria, it is clear that vaccines with improved efficacy are required[14].

An alternative approach to the development of a vaccine against malaria is based on the fact that natural exposure to *Plasmodium* parasites can lead to the development of protective immunity. Attenuated parasites may be used to immunize humans and prevent the development of malaria. The first initiatives to attenuate parasites have been achieved by parasite irradiation (RAS)[15]. More recently, there have been successful attempts to attenuate parasites by genetically engineering techniques resulting in genetically attenuated parasites (GAPs). Studies with GAPs have been shown to produce protective immune responses equal to, or even greater than, those produced by irradiated sporozoites in rodent models[16, 17].

Only one human study with a GAP has been performed, employing *P. falciparum* p52^{-}/p36^{-} [18]. In this first-in-human study, exposure was well tolerated. All volunteers remained blood stage negative after low dose exposure (5 bites/volunteers). Five volunteers remained blood stage negative after high dose exposure, whereas one volunteer developed peripheral parasitemia twelve days after high exposure (200 bites/volunteer). For this reason, the study was discontinued prematurely. All volunteers developed antibodies to CSP. Furthermore, IFN-y response to whole sporozoites and multiple antigens were elicited in 5 of the 6 volunteers, with both CD4 and CD8 cell cytokine production detected. It was concluded that this first generation GAP had favourable immune responses but further development of live, attenuated strains should be pursued.

The disadvantage of using genetically attenuated *P. falciparum* in humans, is the risk of a potential breakthrough of the hepatic stage of the infection. Such a breakthrough may be

avoided by using a *Plasmodium* species that reaches the liver, induces an immune response, but does not result in a breakthrough that causes malaria. The rodent-infective *P. berghei* parasite, is thought to be a suitable candidate for this approach. *P. berghei* is well-known since it has been widely used as a model in malaria research for over 30 years. Cross-species protection between different *Plasmodium* species has been reported in many studies (see Investigator's Brochure). However, immunization of humans by *P. berghei* has never been investigated. The first study investigating the effect of a genetically modified *Plasmodium berghei* in humans is hereby proposed.

To further increase immunogenicity of *P. berghei*, the *P. falciparum* CS protein gene has been added to the *P. berghei* parasite, to significantly enhance the likelihood of protection against *P. falciparum* infection.

1.2.1 Pb(PfCS@UIS4)

The Radboudumc (Nijmegen) malaria group is one of the research groups that are at the forefront of international research on whole organism malaria vaccines worldwide. Radboudumc showed that immunisation with sporozoites administered under chloroquine prophylaxis can induce sustained sterile protective immunity in humans[2, 19]. Radboudumc is an internationally recognized and world leading group in protocol design and execution of a malaria infection (*P. falciparum - Pf)* administered by mosquito bites (Controlled Human Malaria Infections, CHMI; [20, 21]).

An alternative approach in the search of a malaria vaccine could be the use of a genetically modified *P. berghei* parasite. Specifically, the *Pb(PfCS@UIS4)* parasite, in which the Pf circumsporozoite protein (CS) gene has been inserted in the 230p neutral locus of *P. berghei*, in order to express PfCS in addition to the endogenous PbCS.

Plasmodium CS is the immunodominant protective antigen in both RAS and GAS[22]. Previous studies have shown that protection could be achieved by immunization with CS alone [23], a finding that has been at the core of the development of the RTS,S vaccine, presently the most advanced malaria vaccine candidate (reviewed in Casares et al. 2010). However, it is known that CS is not the sole immunogen at play in the immunity triggered by a whole-organism approach. Sterile immunity has been observed in RAS hyper-immunized CSP-transgenic BALB/c mice that are T-cell tolerant to the CSP and cannot produce antibodies [CSP-Tg/JhT(−/−)][24, 25]. This suggests that the observed protection reflects the sum of activities of many minor protective antigens within the RAS.

This will be the first in-human trial of *Pb(PfCS@UIS4)* to evaluate the safety and protective efficacy of the genetically modified parasite.

1.3 Clinical experience with genetically attenuated malaria parasites

One genetically attenuated *P. falciparum* parasite has been evaluated in humans before. The *P. falciparum* GAP Pf∆*p52*∆*p36* is the only GAP so far that has been assessed in humans. Unfortunately, this trial had to be terminated, because of a GAP breakthrough blood infection in one (of six) volunteers in the high dose group, receiving 200 bites, at day 12 post-exposure[18]. It was confirmed that the presence of parasites in the blood was the result of breakthrough blood infection of the genetically-attenuated parasite, not a wild-type parasite. There was no evidence of breakthrough blood infections in the low dose (n=6), five-bite group, or in the other five volunteers from the high dose group.

Local adverse events in the five-bite group of this trial were comparable to those seen in standard CHMI. There were no systemic adverse events through 7 days post-dosing. In the high dose group, all volunteers experienced erythema and pruritus, five experienced oedema (of which one >90mm), one experienced induration, one experienced pain. Systemic adverse events were also reported within 7 days after exposure: fever, malaise (both one volunteer, grade 1), headache (grade 2, two volunteers), nausea and vomiting (2 volunteers, grade 2 in one). Because we aim for a decreased dose (38%), we expect adverse events to be less common and less severe.

1.4 Clinical experience with non-attenuated malaria parasites

There is a large clinical experience with infecting humans by the bites of *P. falciparum* sporozoite-infected mosquitoes. These challenge trials have become highly standardized [21]. 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 [26]. Infecting humans by the bites of *P. falciparum* sporozoite-infected mosquitoes is an established clinical trial methodology and is considered a reproducible, predictable and safe method of inducing *P. falciparum* malaria. The results of such studies were summarized in 1997 [27], in 2007 [28] and in 2012 [29]. Controlled human malaria infections (CHMIs) are accepted as a powerful tool for the evaluation of vaccine and drug efficacy, and the immunology and pathophysiology of early malaria infections [20]. The Radboud university medical center (Radboudumc) and the Erasmus Medical Center/Havenziekenhuis have the experience and

infrastructure to conduct CHMI trials, and conducted two CHMI studies in the past within the same consortium. Over 350 subjects have undergone CHMI in studies conducted by Radboudumc since 2001. Standard operating procedures according to international standards are in place for both clinical and laboratory activities, and the Radboudumc mosquito and parasite culture was positively audited in 2014 by an international independent auditor. Radboudumc has developed a sensitive method of parasite detection by real-time quantitative PCR (18S qPCR) in whole blood that allows us to detect malaria infection in an early stage and is able to detect small differences in parasite density [30].

1.5 Safety

Infection with *Pb(PfCS@UIS4)* is not expected to result in adverse events, other than mild adverse events associated with immunizations or adverse events by reactions to mosquito bites, as this murine *Plasmodium* species cannot multiply in human erythrocytes, which would be required for the onset of pathology. Please refer to D1. Investigator's Brochure for extended safety analyses.

After CHMI, most malaria-naive volunteers experience symptoms such as headache, chills or fatigue during 1-3 days. During the extensive experience with CHMI, severe or life-threatening malaria has never been reported.

In February 2008 a cardiac serious adverse event (SAE) in a twenty-year-old female participating in an LSA3/Alhydrogel (LSA-3 CMO-07/37; NL14715.000.06) malaria vaccine trial was reported to the CCMO (CCMO08.1096/MA/14715). The findings have been published as a case report titled "Cardiac complication after experimental human malaria infection: case report", [31]. The differential diagnosis was acute coronary syndrome or myocarditis but the true nature and pathophysiology of the event remained unclear.

In February 2013, in the TIP5 study (NL39541.091.12), a 23 year old healthy male volunteer experienced a cardiac SAE. The findings have been published as a case report titled "Ideopathic acute myocarditis during treatment for controlled human malaria infection: a case report", [32]. This SAE was diagnosed as myocarditis, while the relation with the malaria infection was not resolved.

In November 2014 a 23 year old healthy male taking part in the BMGF1 study (NL48301.91.14) experienced a cardiac SAE 10 days after a malaria infection under chloroquine prophylaxis (ChemoProphylaxis and Sporozoite immunization - CPS). This SAE concerned an asymptomatic hs-troponin T elevation (maximally 168ng/l) diagnosed as a mild myocarditis as reported to the CCMO (AE001.14.48301). Though certain cardiovascular risk factors were present (smoking, recent cannabis use), the temporal relationship with the

malaria infection and the previous cases of suspected or confirmed myocarditis after malaria infection, make an association with CHMI likely.

As a result of these cardiac SAEs our safety procedures for CHMI have been strongly intensified. In the current trial, we will adhere to those stringent procedures that are relevant, including:

1. Individuals are excluded from participation if they have first or second degree relatives who had cardiac events under the age of 50
2. A positive urine toxicology test for amphetamines, cocaine and cannabis is an exclusion criterion
3. Volunteers who took standard vaccinations within 3 months before the start of the trial or are planning to take standard vaccinations during the trial period up to 8 weeks after CHMI are excluded from participation
4. Volunteers are required to stay close to the Havenziekenhuis to ensure maximal safety from day 5 after CHMI until treatment is finished
5. Increased control of hs troponin T as a marker of cardiac damage; treatment with Malarone® is initiated in consultation with the cardiologist.
6. Daily measurements of platelets; volunteers will be treated when platelet levels are <120x 10^9/L.
7. Curative Malarone treatment after a single positive qPCR (>100 Pf/ml) after CHMI to minimize period of parasitaemia

Transient, asymptomatic liver function derangements have been reported in volunteers in previous CHMI studies, and are likely to be related to the challenge infection (*Reuling et al. manuscript in preparation*). A retrospective analysis of 13 CHMI studies conducted in the Radboudumc showed that 72/120 (60%) of the volunteers that were treated at thick smear parasitaemia levels have mild (38%), moderate (10%) or severe (12%) increases of liver transaminases (ALT/AST). The liver function test (LFT) elevations remained asymptomatic in all the affected volunteers, and are typically not associated with bilirubin changes. Detailed analysis on the LFT elevations pattern showed that these elevations exceeded the upper limit of normal (ULN) around the day of treatment with antimalarials to 2 days after treatment. The peak LFTs elevations are found between 2-14 days after treatment with values normalized at study end (35–42 days after challenge infection). In some volunteers, the elevations were also found associated with lactate dehydrogenase, suggesting that associated sub-clinical haemolysis might have been present during challenge.

Volunteers treated based on qPCR threshold of 100 *Pf*/mL showed a lower percentage, and severity of LFT abnormalities. 13/58 volunteers (22%) showed LFT derangements, with 11/58 (19%) mild, 1/58 (2%) moderate, and 1/58 (2%) severe abnormalities. Importantly, all volunteers showed normalized values at study end.

While severe/complicated malaria is clearly associated with liver damage with sequelae after natural infection (*WHO Severe Malaria , Tropical Medicine and International health 2014)*, such severe complications do not seem to occur in naturally acquired uncomplicated clinical malaria (*Abro et al. J Coll Physicians Surg Pak. 2009 and Ghoda et al. Trop Gastroenterol. 2002*). In this context it is important to note that parasite densities under these conditions are several logs higher than observed in the CHMI model.

A clear explanation for the transient elevated transaminasesin our CHMI studies is not obvious. Although higher parasitaemia associates with higher chances of LFT elevations on group level, there is no clear relationship on individual levels. Given the diversity of drug regimens used, it also seems unlikely to be directly related to anti-malarials. Similarly, the timing, differential, and limited use of paracetamol does not support a clear relationship. Rather a combination of the above mentioned factors, and individual susceptibility may have triggered the observed abnormalities.

Following an independent review of the safety committee, and a hepatology expert, it was concluded that these transient asymptomatic and severe LFT elevations are most likely a direct consequence of the malaria infection rather than reflecting a direct drug-induced liver injury caused by the antimalarial drug or other liver noxe. Given the rapid and spontaneous resolution of the observations and the absence of significantly elevated bilirubin these events were judged to not cause permanent subclinical liver damage, and to not preclude further studies utilising *Plasmodium falciparum* challenge.

As a result of the LFT elevations our safety procedures for CHMI have been intensified. In the current trial, we will adhere to those stringent procedures that are relevant, including:

1. Volunteers will undergo regular safety monitoring to assess asymptomatic liver function test abnormalities.
2. Avoid additional triggers that may cause elevations in liver enzymes including alcohol from baseline up to 1 week post treatment.
3. Maximum dose of 3000 mg per day of paracetamol/acetaminophen
4. Initiation of a curative antimalarial treatment at a ALT/AST levels >5x ULN

In this study, volunteers will be treated on a qPCR threshold of 100 *Pf*/mL after CHMI.

Furthermore, transient, slight decreases in renal function was observed during CHMI. Therefore, extra safety measurements are included. In the current trial, we will adhere to those stringent procedures that are relevant, including:

1. Exclusion of subjects with abnormal renal function at baseline.
2. Upon inclusion: Avoid additional triggers that may cause renal dysfunction
    a. Alcohol intake from baseline up to 1 week post-treatment
    b. Heavy physical exercise
    c. Party drugs
3. Standard monitoring of renal function at day *C-1; C+6; TD, TD+2, C+35.*

2. OBJECTIVES

Phase 1

Primary objective:

To determine the safety and tolerability of administration of *Pb(PfCS@UIS4)* to healthy volunteers delivered by infectious mosquito bite.

Exploratory objective:

To analyse immune responses in volunteers immunized by *Pb(PfCS@UIS4)* and challenged by *P.falciparum*.

Phase 2

Primary objective:

To determine the safety, tolerability and protective efficacy of immunization with *Pb(PfCS@UIS4)* against Controlled Human Malaria Infection by mosquito bite.

Exploratory objective:

To analyse immune responses in volunteers exposed to *Pb(PfCS@UIS4)* and challenged by *P.falciparum,* and to determine cytokine responses, coagulation activation and inflammation parameters in non-immune individuals(controls) infected with *P. falciparum* during malaria liver phase.

3. STUDY DESIGN

This is a multicenter, open label, adaptive design study, and will be divided in two phases.
Volunteers will be recruited and screened in the Havenziekenhuis in Rotterdam. Administration of *Pb(PfCS@UIS4)* to human volunteers will take place in the Radboudumc in Nijmegen. After inoculation, volunteers will be monitored in the Havenziekenhuis. Clinical chemical and microbiological analysis and storage of the samples of the infected volunteers will be performed in laboratories of the Erasmus MC in Rotterdam. Some biochemical and microbiological analyses will be performed in the Radboudumc. Please see figure 1 for study flow and activities of the centers.

Phase 1, will be a multicenter, dose escalation trial. There will be no control group in this phase.
A total of eighteen healthy adult volunteers will be recruited and divided over 3 groups. Three volunteers (Group 1) will be exposed to bites of five *Pb(PfCS@UIS4)*-infected mosquitoes. Volunteers will be closely monitored for adverse events for a period of 28 days. If safe on day 14, another group of three volunteers (group 2) will be exposed to 25 *Pb(PfCS@UIS4)*-infected mosquito bites. Finally, on day 14 of group 2, if considered safe, a group of 12 volunteers (group 3) will be exposed to 75 *Pb(PfCS@UIS4)*-infected mosquito bites (n=12). If a safety issue or a stop criterion is met in the previous group after day 14, the following group will immediately be treated. These volunteers will visit the study centre once daily from day 1 until day 10 after exposure and every other day from day 12 until day 20 after exposure. If one of the volunteers is not fit to participate in the study on day 0, an alternate volunteer will replace him/her. For this purpose one additional volunteer will be screened for possible back up in group 1 and 2 and two additional volunteers will be screened for possible back up for group 3.

All exposed volunteers will be treated with a curative regimen of Malarone (atovaquone/proguanil), either at the time of detection of blood stage parasitemia by thick smear or 28 days after exposure to *Pb(PfCS@UIS4)* infected mosquitoes for group 1 and 2. Volunteers of group 3 will subsequently enter phase 2, if phase 1 (until day 28) is considered safe and if no blood stage parasitemia is detected during follow up. End of follow up will be 100 days after exposure to *Pb(PfCS@UIS4)* for groups 1 and 2.
Depending on the results of Phase 1, Phase 2 will commence. Criteria for proceeding to the second phase are described in section 8.4.4.

Phase 2, will be a multicenter, safety and efficacy trial with an adaptive design method. A control group will be included for the challenge phase. The trial objective is to evaluate the safety and protective efficacy of repeated exposure to *Pb(PfCS@UIS4)*-infected mosquito bites by subsequent controlled human malaria infection (CHMI) of test subjects. The same volunteers of group 3 will be exposed three additional times to the bites of 75 *Pb(PfCS@UIS4)*-infected mosquitoes *(n=12),*at four, four, and four to eight week interval. Four weeks after the last exposure all volunteers will undergo a CHMI with 5 *P. falciparum* (NF54)-infected mosquitoes. Six infectivity control subjects will be recruited to receive 5 *P. falciparum* (NF54) infective mosquito bites per challenge infection.

Based on the first results of phase 2 an *ad hoc* decision will be made to include a potential booster immunization and/or subsequent second challenge infection with five *P. falciparum* (NF54)-infected mosquitoes. The booster immunization and/or subsequent second challenge infection will be initiated using the following criteria:

1. In case 2 to 4 volunteers are sterilely protected, all volunteers will receive a booster immunization followed by a second challenge infection, 3 months after the booster immunization.
2. In case ≥5 volunteers are sterilely protected, all non protected volunteers will receive a booster immunization followed by a second challenge infection 3 months later; all protected volunteers will only receive a second challenge infection to assess durability of protection.

All exposed volunteers are subjected to close follow-up after exposure, with regular visits to the clinical trial centre at the Havenziekenhuis in Rotterdam, periodic physical examinations, frequent blood sampling and adverse events will be recorded in a diary. For all subjects, during this period, all relevant investigations will be carried out on an outpatient basis, including frequent safety analyses.

Exposed volunteers will be treated with a curative regimen of Malarone (atovaquone and proguanil), at the time of detection of blood stage parasitemia by thick smear during immunizations. During the challenge infection, all volunteers will receive a curative treatment, either at the time of blood stage parasitemia by qPCR or 28 days post CHMI. Follow up will end 100 days after the last exposure to *Pb(PfCS@UIS4)* and 35 days post CHMI for the infectivity control subjects. Depending on the results of the immunizations of phase 2, the volunteers will receive a CHMI. Criteria for proceeding to CHMI are described in section 8.4.4.

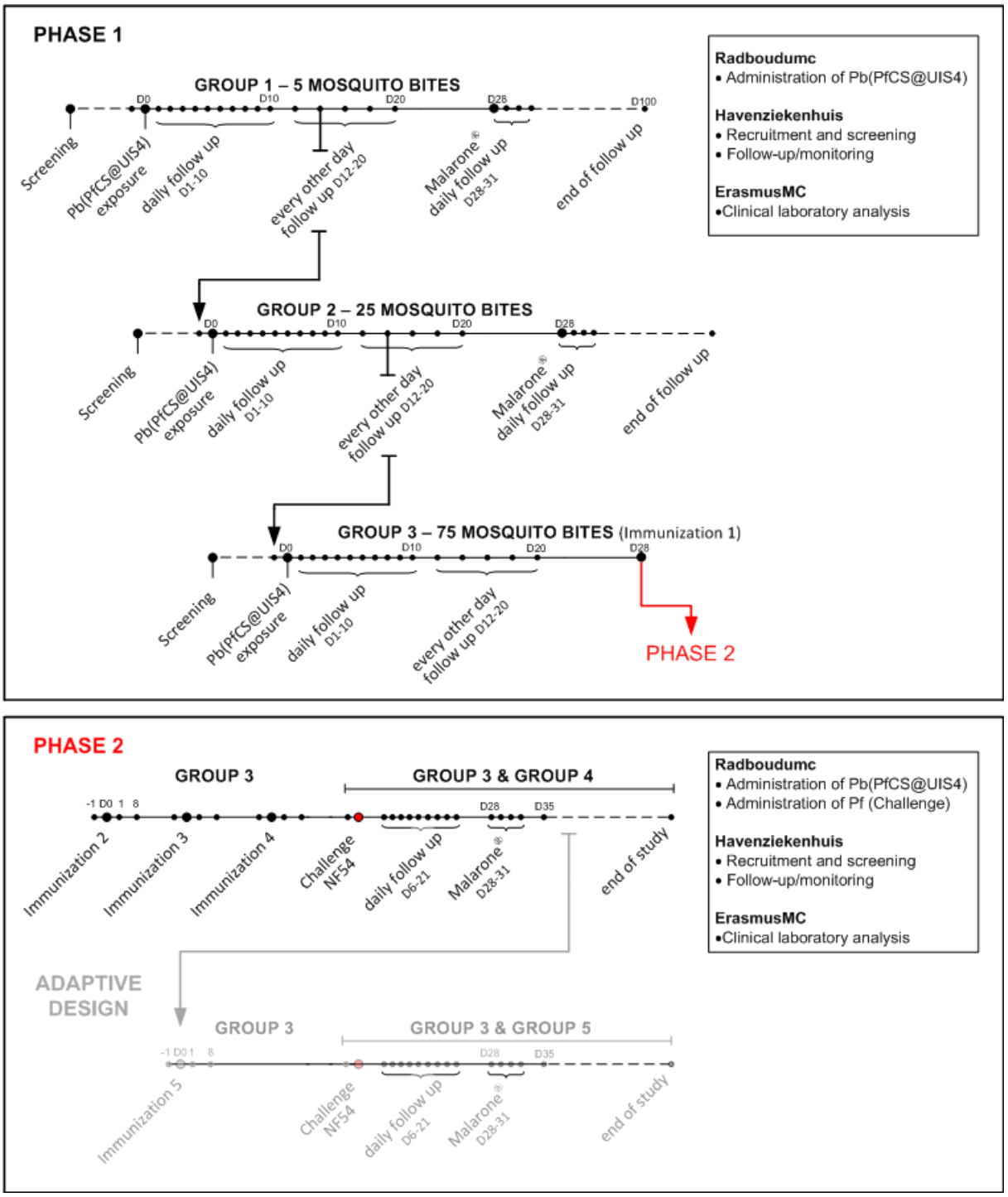

Figure 1: Study schedule of Phase 1 and Phase 2

4. STUDY POPULATION

4.1 Population (base)

The study population will be comprised of healthy adult male and female, malaria-naïve subjects. A maximum of 30 subjects will be enrolled to participate in the study. The

investigator will ensure that all subjects being considered for the study meet the following eligibility criteria. Subject 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.

4.2 Inclusion criteria

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

1. Subject is aged ≥ 18 and ≤ 35 years and in good health.
2. Subject 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.
3. Subject is willing to complete an informed consent questionnaire and is able to answer all questions correctly.
4. Subject is able to communicate well with the investigator and is available to attend all study visits, lives in Rotterdam or in proximity to the trial centre (can be on site within 1 hour) or is willing to stay in a hotel close to the trial centre during part of the study (phase 1: from day of immunization to day 12 post-immunization; phase 2: from day of immunization to day 8 post-immunization.
5. The subject will remain within the Netherlands from day -1 until day +28 after immunization during phase 1; from day -1 until day 12 after each immunization during phase 2, and during the challenge period, will not travel to a malaria-endemic area during the study period, and is reachable (24/7) by mobile telephone throughout the entire study period.
6. Subject agrees to their general practitioner being informed and contacted about their participation in the study and agrees to sign a form to request the release by their General Practitioner (GP), and medical specialist when necessary, to the investigator(s), of any relevant medical information concerning possible contra-indications for participation in the study.
7. The subject agrees to refrain from blood donation to Sanquin or for other purposes throughout the study period and for a defined period thereafter according to current Sanquin guidelines (3 years minimum, depending on serology).
8. For female subjects: subject agrees to use continuous adequate contraception** and not to breastfeed for the duration of study.
9. Subject agrees to refrain from intensive physical exercise (disproportionate to the subject's usual daily activity or exercise routine) during the malaria challenge period.

10. Subject agrees to avoid additional triggers that may cause elevations in liver enzymes including alcohol from baseline up to 1 week post treatment.
11. Subject has signed written informed consent to participate in the trial.

*(*Acceptable forms of contraception include: established use of oral, injected or implanted hormonal contraceptives; intrauterine device or intrauterine system; barrier methods (condoms or diaphragm with additional spermicide); male partner's sterilisation (with appropriate post-vasectomy documentation of absence of sperm in the ejaculate); true abstinence when this is in line with the preferred and usual lifestyle of the subject; Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.)*

4.3 Exclusion criteria

A potential subject 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 volunteer 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. A heightened risk of cardiovascular disease, as determined by: an estimated ten year risk of fatal cardiovascular disease of ≥5% at screening, as determined by the Systematic Coronary Risk Evaluation (SCORE); history, or evidence at screening, of clinically significant arrhythmia's, prolonged QT-interval or other clinically relevant ECG abnormalities; or a positive family history of cardiac events in 1st or 2nd degree relatives <50 years old.
    1.3. A medical history of functional asplenia, sickle cell trait/disease, thalassaemia trait/disease or G6PD-deficiency.
    1.4. History of epilepsy in the period of five years prior to study onset, even if no longer on medication.
    1.5. Screening tests positive for Human Immunodeficiency Virus (HIV), active Hepatitis B Virus (HBV), Hepatitis C Virus (HCV)

1.6. Chronic use of i) immunosuppressive drugs, ii) antibiotics, iii) or other immune modifying drugs within three months prior to study onset (inhaled and topical corticosteroids and oral anti-histamines exempted) or expected use of such during the study period.

1.7. Any recent or current systemic therapy with an antibiotic or drug with potential anti-malarial activity (chloroquine, atovaquone-proguanil, arthemether-lumefantrine, sulfadoxine-pyrimethamine, doxycycline, tetracycline, piperaquine, benzodiazepine, flunarizine, fluoxetine, tetracycline, azithromycin, clindamycin, erythromycin, hydroxychloroquine, etc.) (allowable timeframe for use at the Investigator's discretion).

1.8. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years.

1.9. Any history of treatment for severe psychiatric disease by a psychiatrist in the past year.

1.10. History of drug or alcohol abuse interfering with normal social function in the period of one year prior to study onset, positive urine toxicology test for cocaine or amphetamines at screening or at inclusion, or positive urine toxicology test for cannabis at inclusion.

2. For female subjects: positive urine pregnancy test at screening and/or at the baseline visits, including baseline of immunizations (I-1) and or baseline before CHMI (C-1).
3. Any history of malaria, positive serology for *P. falciparum*, or previous participation in any malaria (vaccine) study.
4. Known hypersensitivity to or contra-indications (including co-medication) for use of sulfadoxine-pyrimethamine, piperaquine, chloroquine, Malarone®, artemether-lumefantrine, primaquine or history of severe (allergic) reactions to mosquito bites.
5. Receipt of any vaccinations in the 3 months prior to the start of the study or plans to receive any other vaccinations during the study period or up to 8 weeks thereafter.
6. Participation in any other clinical study in the 30 days prior to the start of the study or during the study period.
7. Being an employee or student of the department of Medical Microbiology and Infectious Diseases of the Erasmus MC or Radboudumc, or the department of Internal Medicine of the Radboudumc or Havenziekenhuis.
8. Any other condition or situation that would, in the opinion of the investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

4.4 Sample size calculation

Phase 1 (safety trial) is a first in-human observational trial, for which no formal power calculation has been performed. Based on previous experience and convention, we will start with a small group of three volunteers and subsequently expand to a group of twelve volunteers. The occurrence of (rare) AEs will follow a Poison distribution and the proposed sample size will only allow a crude estimation of confidence intervals. If, for example, 1 individual (out of 12) would experience an AE, the 95% confidence interval would have an upper limit of 46.4%, indicating that an actual risk exceeding this percentage is ruled out with 95% confidence. Previously performed phase 1 dose-escalation trials with malaria candidate vaccines used similar population sizes and examined the risk of AEs with more precision in follow-on trials. We envisage a similar route for the current intervention where the current trials form an essential first assessment of safety in a small, intensively monitored population.

Phase 2 (efficacy trial) is a pilot study designed to provide proof-of-concept for the induction of sterile protection by transgenic *P. berghei (Pb(PfCS@UIS4)*) parasites against malaria challenge infection with the *P. falciparum* NF54 strain. From 13 previous CHMIs with NF54 we know that the time to positive qPCR with >100 parasites/ml after a single challenge infection is on average 7.7 days, with a standard deviation of 1.46 days. In order to detect a difference in pre-patent period of 2.5 days between intervention and control groups (α=0.05, 1-ß=0.80) a sample size of 10 volunteers in the treatment group and 5 volunteers in the control group is required. Correcting for a possible withdrawal rate of 2 volunteers in group 3, and 1 volunteer in the control group, we therefore chose a sample size of 12 volunteers in the intervention group and 6 volunteers in the control group.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

No investigational products are applied during this study.

5.2 Use of co-intervention (if applicable)

In phase 1, all exposed volunteers of group 1 and 2 will be treated with a curative regimen of Malarone® (atovaquone/proguanil), either at the time of detection of blood stage parasitemia by thick smear or 28 days after exposure to *Pb(PfCS@UIS4)* infected mosquitoes. Group 3 will only be treated in phase 1 if a blood stage parasitemia occurs as detected by thick smear.

In phase 2, all volunteers will be treated with a curative regimen of Malarone® (atovaquone/proguanil) as soon as blood stage parasitemia is detected by thick smear (during the immunizations period) or qPCR (during CHMI) at any point in the trial or on day 28 following CHMI.

Pre-emptive rescue treatment with Malarone® can commence whenever deemed necessary by the investigator.

The following list of concomitant medications or conditions are contraindications to atovaquone/proguanil, based on the product monograph.

Concomitant medications:

- Use of anticoagulants (warfarin and other coumarin based anticoagulants).
- Use of rifampicin, rifabutin, tetracycline or metoclopramide.
- Use of indinavir.

5.3 Escape medication (if applicable)

Volunteers are advised to take paracetamol for complaints secondary to the CHMI (fever, muscle aches, headache, etc.). Paracetamol or any other symptomatic treatment will be supplied to the volunteers without charge. The maximum dose of paracetamol is 4 grams a day.

Although volunteers with a previous history of severe reactions to mosquito bites will be excluded from the study, some volunteers may experience local reactions to the mosquito bites and require topical and/or oral medications. Topical tripelennamine and topical steroids such as 1% hydrocortisone cream and for more severe reactions, clobetasol 0.05% cream

will be available in the clinical trials center for distribution to the volunteers. Due to the possible, yet unknown, effects on sporozoite migration from the skin in an area treated with steroids, volunteers will be asked to wait 2 hours after biting prior to applying steroid cream to affected areas. Additional medications available to volunteers by the clinical investigators will be oral cetirizine or other anti-histamines. Volunteers with exuberant reactions to mosquito bites may receive cetirizine prophylactically before immunization to abrogate skin reactions such as redness, swelling and itching.

6. INVESTIGATIONAL AND NON-INVESTIGATIONAL PRODUCT (CHALLENGE PRODUCT)

Volunteers will be exposed to the bites of five, 25 or 75 *Pb(PfCS@UIS4)*-infected mosquitoes on day 0 of the study in phase 1. In phase 2, volunteers will receive three additional immunizations, and a possible 5th booster immunization with *Pb(PfCS@UIS4)*, and/or a second challenge infection depending on the outcome of the first controlled challenge infection (see also **section 3**). Five *P. falciparum* NF54-infected *Anopheles stephensi* mosquitoes will be used for the purpose of controlled human malaria infections (CHMI).

Pb(PfCS@UIS4 is a genetically modified *P.berghei* parasite line in which the *Plasmodium falciparum* CS protein gene has been inserted.

6.1 Name and description of non-investigational products

6.1.1 Name and description of investigational product (*Pb(PfCS@UIS4))*

A genetically modified *P. berghei* parasite is used in this study, the *Pb(PfCS@UIS4)* parasite, where the PfCS gene has been inserted in the 230p neutral locus of *P. berghei* and that, therefore, expresses PfCS in addition to the endogenous PbCS.

6.1.2 Name and description of non-investigational product (*P. falciparum* NF54)

P. falciparum isolate NF54 infected *Anopheles stephensi* mosquitoes for the purpose controlled human malaria infection (CHMI).

6.2 Summary of findings from non-clinical studies

6.2.1 Summary of findings from non-clinical studies (*Pb(PfCS@UIS4))*

Pb(PfCS@UIS4) has been extensively investigated in non-clinical studies with (chimeric) mice, rabbits and Rhesus monkeys. The first milestones of the proof-of-principle of the proposed strategy have recently been completed through validation of the three core premises. 1) *Pb(PfCS@UIS4)* can infect and develop in human hepatocytes. 2) The parasite is unable to develop inside human erythrocytes. 3) Immunization with *Pb(PfCS@UIS4)* can elicit immune responses against *P. falciparum*.

The non-clinical studies have demonstrated that the hepatic and mosquito infectivities of *Pb(PfCS@UIS4)* are similar to those of the wild-type parental *P. berghei* strain, showing that

the genetic modification introduced did not in any way impair the resulting parasite's sporogonic development or ability to infect rodent hepatic cells in vitro, ex vivo and in vivo. The non-clinical data have further shown that *Pb(PfCS@UIS4)* parasites are able to infect and develop in vitro inside human hepatoma cells and human immortalized hepatocytes, as well as in vivo in human hepatocytes in chimeric (liver-humanized) mouse livers, warranting the basis for the proposed immunization approach in humans. The non-clinical studies have also shown that *Pb(PfCS@UIS4)* is unable to develop inside human erythrocytes in a blood-humanized mouse model, which supports its safety for use in humans, in full alignment with the widely accepted notion that this is a non-pathogenic organism for humans. Nevertheless, the drug sensitivity studies further showed that, in the extremely unlikely event of a breakthrough infection by *Pb(PfCS@UIS4)*, the blood stages of this parasite are sensitive to and can be completely eliminated by treatment with the anti-malarials Malarone© and Coartem©.

Moreover, the non-clinical studies showed that the proposed strategy can also work in non-natural hosts, such as rabbits, and Rhesus monkeys. These studies have shown that *Pb(PfCS@UIS4)* can infect and develop inside rabbit-, and Rhesus monkey- hepatocytes, both *ex vivo* and *in vivo*. Crucially, they have also shown that *Pb(PfCS@UIS4)* is unable to cause a blood-stage infection in rabbits or Rhesus monkeys, neither when infection is initiated by sporozoite injection nor when it is initiated by the injection of as many as 100 million infected red blood cells in rabbits.

Importantly, the non-clinical program also showed that immunization of mouse models with *Pb(PfCS@UIS4)* elicits *P. berghei/P. falciparum* cross-protective, as well as *P. falciparum* CS-specific cellular immune responses. Immunization experiments employing both mice and rabbits further showed that *Pb(PfCS@UIS4)* elicits *P. falciparum* CS-specific humoral responses capable of recognizing *P. falciparum* sporozoites. Furthermore it inhibits a *P. falciparum* infection of human immortalized hepatocytes and of human hepatocytes in chimeric mouse livers. The data also showed that infection with *Pb(PfCS@UIS4)* induces Type I IFN pathways in both mouse and human hepatocytes of chimeric mice. Finally, the data showed that immunization of monkeys with *Pb(PfCS@UIS4)* elicits strong immune responses against *Pf*CS protein and *P. falciparum* sporozoites, clearly indicative of vaccine-take and immunogenicity of *Pb(PfCS@UIS4)*.

Furthermore, biodistribution studies, drug sensitivity testing and a repeated-dose toxicology study have recently been completed.

Overall, the non-clinical program provides solid evidence that the parasite proposed to be used in this study fulfills all the safety requirements for human use, as it is highly unlikely to cause disease in humans. Besides, the parasite also meets the efficacy requirements to be used as a vaccine candidate, as it was shown to successfully infect human hepatocytes, a requirement for immunity generation by this type of vaccine candidate. Finally, the non-clinical data shows that the parasite is able to induce immune responses that are able to recognize and inhibit a *P. falciparum* infection.

Please refer to the Investigator's Brochure for detailed description of all the non-clinical studies.

6.2.2 Summary of findings from non-clinical studies (*P. falciparum* NF54)

The *P. falciparum* NF54 strain was isolated from a patient living in the Schiphol-area in the Netherlands. In *in vitro* studies, this parasite has been shown completely susceptible to multiple antimalarials, including chloroquine, mefloquine, atovaquone/proguanil and arthemeter/ lumefantrine (see D2c, 'Product information *Plasmodium falciparum* infected *Anopheles* mosquitoes').

6.3 Summary of findings from clinical studies

6.3.1 Summary of findings from clinical studies (*Pb(PfCS@UIS4))*

This is the first trial of *Pb(PfCS@UIS4)* in humans. Therefore, no clinical studies are available yet.

6.3.2 Summary of findings from clinical studies (*P. falciparum* NF54)

CHMIs are well accepted as a powerful tool for the evaluation of parasite development in humans. The Radboudumc, ErasmusMC and the Havenziekenhuis have the experience and infrastructure to conduct CHMIs. The Radboudumc has developed the very sensitive method of parasite detection by real-time qPCR available that will allow us to detect malaria infection in an early stage, providing early treatment.

There is a large clinical experience with infecting humans by the bite of *P. falciparum*-infected mosquitoes. Since 1986 more than 3,500 volunteers have had a CHMI by the bites of mosquitoes fed on cultures of *P. falciparum* gametocytes to produce *P. falciparum* sporozoites [26]. This has proved to be a reproducible, predictable and safe method of

inducing *P. falciparum* malaria. The results of such studies were summarized in 1997 [27], in 2007 [33] and in 2012 [29].

The *P. falciparum* isolate NF54 has been tested for the purpose of challenge infection in 353 volunteers in 22 CHMI studies in the Radboudumc and 2 studies in collaboration with the ErasmusMC and the Havenziekenhuis. In these studies generally all malaria-naive volunteers developed patent parasitemia after bites from five NF54 infected mosquitoes. The median time to blood stage parasitemia detectable by qPCR was 7.0 days. Mild-moderate solicited adverse events were generally experienced by all study subjects post-infection, most common were headache (95%), general malaise/fatigue (65%) and fever (90%). Gastro-intestinal complaints, including nausea, diarrhoea and abdominal pain were experienced by several subjects, mainly following intake of atovaquone-proguanil. Symptoms that were severe enough to prohibit daily activities occurred in 49% of participants. All volunteers have been successfully treated with anti-malarials.

6.4 Summary of known and potential risks and benefits

6.4.1 Summary of known and potential risks and benefits of *Pb(PfCS@UIS4)* immunization

There is no benefit expected for subjects participating in this study. The risk to subjects after immunization with *Pb(PfCS@UIS4)*-infected mosquitoes in this trial will be minimized by adherence to the inclusion/exclusion criteria and close clinical monitoring, which ensures that subjects with a 'break-through' blood stage infection will be treated at earliest stages of parasitemia. The risks of a CHMI for malaria-naïve subjects include i) discomfort induced by mosquito bites, ii) discomfort associated with periodic blood drawing and iii) risk of acquiring a blood stage parasitemia with *Pb(PfCS@UIS4).*

Mosquito bites are known to cause mild discomfort associated with mosquito feeding. A small amount of inflammation and pruritus typically accompanies the bite of the insect. It is possible that some subjects will experience local skin induration. Anaphylaxis to the bite of a mosquito is extremely rare and has never been reported after CHMI. While significant allergic reactions are extremely rare, in the event of an allergic reaction, topical steroids, anti-histamines, epinephrine, on-call physician and resuscitation equipment are available on site. The Radboudumc, an established site for CHMIs, is fully equipped to manage anaphylaxis and any other medical emergency.

Frequent blood draws will be necessary to closely monitor the subjects and to perform thick smears for early detection of blood stage parasitemia. Universal precautions will be maintained for the protection of the volunteer and the study personnel. The total amount of blood collected will be maximally 1500 ml over the entire trial period (including screening

period and potential 5th booster immunization and/or 2nd challenge infection), in agreement with guidelines of the Sanquin blood bank.

Intensive follow-up with thick smears performed on samples taken once daily will allow for detection of parasites at a very early stage. As therapy will be initiated at this early stage, dangerously high levels or prolonged duration of parasitemia that would put the subject at undue risk, will not occur. Researchers at the Radboudumc and the ErasmusMC and Havenziekenhuis have extensive experience with the care of clinical malaria.

Malarone® (atovaquone-proguanil) is effective against *Pb(PfCS@UIS4),* as tested pre-clinically, and is a marketed first-line treatment for malaria and will be used. Furthermore, Malarone® is generally well tolerated. Common adverse reactions (≥5%) in adults associated with Malarone® treatment include abdominal pain, nausea, vomiting, headache, diarrhoea, asthenia, anorexia, and dizziness. Those volunteers intolerant to Malarone® will be given alternative treatment according to the national malaria treatment guidelines, such as arthemether-lumefantrine.

The exposure to *Pb(PfCS@UIS4)* -infected mosquito bites will occur at Radboudumc insectary, which has a double-door barrier system along with a double blower (negative pressure wind blockade) to prevent mosquito flight across entryways.

6.4.2 Summary of known and potential risks and benefits of *Pf* challenge

There is no benefit expected for subjects participating in this study. The risk to subjects after challenge infection with *P. falciparum* NF54-infected mosquitoes 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 parasitaemia. The risks of a CHMI for malaria-naïve subjects include i) discomfort induced by mosquito bites, ii) discomfort associated with periodic blood drawing and iii) risk of acquiring mild clinical *P. falciparum* malaria.

Mosquito bites are known to cause mild discomfort associated with mosquito feeding. A small degree of inflammation and pruritus typically accompanies the bite of the insect. Anaphylaxis after mosquito bites is extremely rare and has never been reported after CHMI. While significant allergic reactions are extremely rare, in the event of an allergic reaction, epinephrine, anti-histamines, on-call physician and resuscitation equipment are available on site. The Radboudumc, an established site for CHMIs, is fully equipped to manage anaphylaxis and any other medical emergency.

Frequent blood draws will be necessary to closely monitor the subjects and to perform qPCR for detection of *P. falciparum* parasitaemia. Universal precautions will be taken for protection of the volunteer and study personnel. The total amount of blood collected will be maximally 1500 ml over the entire trial period (including screening period and potential 5th booster

immunization and/or 2nd challenge infection), in agreement with guidelines of the Sanquin blood bank.

Intensive follow-up with qPCR performed on samples taken once daily, will ensure early treatment - preventing high levels or prolonged duration of parasitaemia that would put the subject at undue risk. Severe/complicated malaria has never been described in a CHMI. Mild malaria symptoms include headache, myalgia, fever, chills, sweats, nausea, vomiting, and diarrhoea. Researchers at the Radboudumc have extensive experience with the care of clinical malaria.

Although subjects often become symptomatic with mild malaria after CHMI, rapid diagnosis by qPCR and/or thick smears and subsequent treatment quickly attenuates the illness so that the infection does not place the subject at undue risk.

The exposure to infected *P. falciparum* NF54-infected mosquito bites will occur at Radboudumc insectary which has a double-door barrier system along with a double blower (negative pressure wind blockade) to prevent flight across entryways.

6.5 Description and justification of route of administration and dosage

6.5.1 Description and justification of route of administration and dosage (*Pb(PfCS@UIS4))*

Pb(PfCS@UIS4) will be administered through the bites of infected *Anopheles stephensi* mosquitoes.

Being the first in-human trial of *Pb(PfCS@UIS4)*, three different doses were chosen. The first dose (5 mosquito bites) allows for optimal safety as it is a dose most commonly used for CHMI trials worldwide. This will allow for a very safe first evaluation of the phenotype. If uneventful, the dose is increased to 25 bites and, if safe, subsequently to 75 bites, which was chosen as the lowest dose that has the potential to induce immunity in subsequent immunisation trials based on data from comparable trials with non-attenuated sporozoites under chloroquine prophylaxis (CPS) and radiation-attenuated sporozoites (RAS). CPS is known to induce immunity in a subset of volunteers at a total dose of 24 mosquito bites, complete protection at a dose of 45 bites. RAS is likely to induce immunity in a subset of volunteers at cumulative doses >200 bites and full immunity at doses >1000 bites[8]. CPS is thought more efficient than *Pb(PfCS@UIS4)*, because it allows for the complete liver stage development of *P. falciparum* parasites (and thus *Pf*-specific antigenic exposure), whereas RAS is though less efficient than *Pb(PfCS@UIS4)* because sporozoites will arrest very early in their development. With a dose of 75 bites, we envision that we will achieve a dose of >200 infectious bites in trials with 4 immunizations, which we believe should be sufficient to

obtain proof-of-concept. The potential 5th dose will function as a booster immunization for the volunteers that are not or only partially protected after the 4th immunization.

6.5.2 Description and justification of route of administration and dosage (*P. falciparum* NF54)

Study subjects will be exposed to malaria for the purpose of challenge infection through (cutaneous) bites of *P. falciparum* NF54-infected female *Anopheles stephensi* mosquitoes. This is the natural route of infection and the one with which most experience has been accumulated in CHMI trials. Volunteers will be exposed to the bites of 5 *P. falciparum* NF54 infected mosquitoes, which is the gold-standard dosage for CHMI studies at the Radboudumc, based on extensive experience with the *P. falciparum* NF54 strain.

6.6 Dosages, dosage modifications and method of administration

6.6.1 Dosages, dosage modifications and method of administration (*Pb(PfCS@UIS4)*)

Please also refer to paragraph 6.5.1. Because, at time of post-immunization dissection, not all 75 *A. stephensi* mosquitoes will be found to be infected with sporozoites and/or will not have taken a blood meal, a higher number of mosquitoes will be used at each immunization time point to achieve an end result of 75 infectious bites. Based on a pre-clinical repeated-dose toxicity study the average mosquito feeding rate was 80.5%, and the average mosquito mortality 4%. Therefore, 97 mosquitoes will be used at each immunization time point to obtain the targeted 75 infected mosquito bites. The mosquitoes will be placed in a container and allowed to feed for 15 minutes. All mosquitoes will be checked for blood meal assessment. When the number of blood-fed mosquitoes is below <65, <55, or <45 the volunteer will be exposed to extra bites of 10, 20 or 30 infected- mosquitoes respectively. This method of immunization will enable investigators to monitor that volunteers do receive approximately 75 infectious bites at each immunization, although the actual bite number may be slightly more or less.

6.6.2 Dosages, dosage modifications and method of administration (*P. falciparum* NF54)

See also paragraph 6.5.2. Subjects receiving a challenge infection with the *P. falciparum* NF54 strain will receive 5 infectious bites from *A. stephensi* mosquitoes infected with *P.*

falciparum NF54 sporozoites. Previous studies have demonstrated that with this dosage, near 100% of malaria-naïve volunteers develop a blood stage malaria infection [34].

6.7 Preparation and labelling of Investigational, Non Investigational Medicinal Product

6.7.1 Preparation and labelling of Investigational Medicinal Product (*Pb(PfCS@UIS4))*

The culture of parasites and infection of mosquitoes has been a routine procedure for over 20 years in the Malaria Unit of the Central Animal Facility of the Radboudumc, Nijmegen.
In vivo cultures of *P. berghei* parasites and Pb-infected mosquitoes have been extensively produced in the Malaria Unit of the Radboudumc, Nijmegen. Specifically, *Pb(PfCS@UIS4-*infected mosquitoes have already extensively been produced for the toxicology study by the Radboudumc.

A quality control protocol of the sporozoites used for immunization in human trials is outlined in Figure 2. It includes **(I)** the creation of a Master Bank of parasites, with complete microbiological and genetic analysis of the samples stored there (see Master parasite stocks control below). **(II)** The use of SPF mice, subjected to complete microbiological analysis, which will be infected with Master Bank aliquot (see Animal quality control: SPF mice below). **(III)** The collection of salivary gland sporozoites injected by infected mosquitoes, followed by genetic analysis to characterize the parasite-strain identity by PCR and exclude possible contamination. The whole cycle will be performed at least once before the actual trial and the analysis in **(II)** and **(III)** will be repeated on the actual batch to be used in the trial.

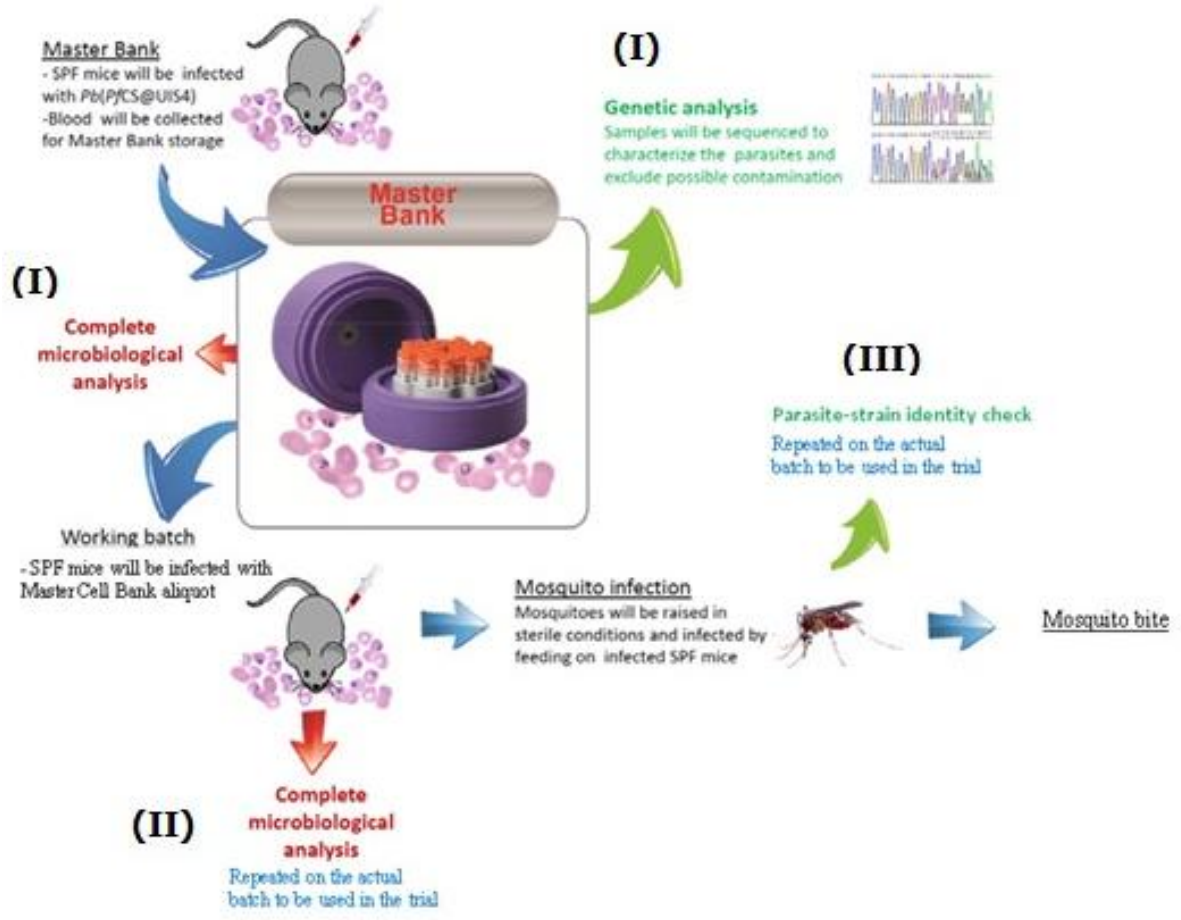

Figure 2 Quality control plan for immunizing sporozoite material.

Any culture material or cages with mosquitoes that contain genetically attenuated *Pb(PfCS@UIS4)* parasites will be labelled with a bright yellow label (Section D3).

Please refer to *D2a IMPD of Pb(PfCS@UIS4)* and *D2i Background Information Pb(PfCS@UIS4) infected Anopheles mosquitoes* for a detailed description of the generation and production of *Pb(PfCS@UIS4) and Pb(PfCS@UIS4)*- infected *Anopheles* mosquitoes.

6.7.2 Preparation and labelling of Non Investigational Medicinal Product (*P. falciparum* NF54)

The culture of parasites and infection of mosquitoes has been a routine procedure for over 20 years in the Malaria Unit of the Central Animal Facility of the Radboudumc, Nijmegen. The *P. falciparum* NF54 isolate originates from the Schiphol area. The isolate was originally derived from patient material and was cultured in vitro in RPMI-1640 medium with 10% serum and 5% haematocrit red blood cells. Both the serum and the red blood cells are obtained from the Nijmegen department of the Sanquin Bloedbank region Zuid-Oost. Both are negative for malaria and Hepatitis B surface Anitgen (HBsAg), and seronegative for HIV,

HCV, HumanT-lymphotropic Virus (HTLV) 1+2 and syphilis. The cultures are checked for bacterial, fungal and *Mycoplasma* contamination.

To produce infectious gametocytes, the asexual parasites will be cultured *in vitro*. After 14 days of culture, the sexual stage parasites will be harvested for feeding to 1-5 days old laboratory cultured *Anopheles stephensi* mosquitoes via a 'membrane feeder'. The percentage *P. falciparum*-infected mosquitoes will be assessed 6-9 days after feeding and one day prior to the CHMI.

Mosquitoes are kept in the same midi-cage from Membrane Feed for Sporozoite production (MFS) until the day before CHMI. Mosquitoes infected with different strains are kept in different midi-cages, labeled with distinguishable coloured labels identifying the study, the strain, the date of MFS, and the feeder-number. On the day before CHMI, a sample of 10 mosquitoes from each batch is checked for the presence of sporozoites and a sample of 10 mosquitoes is assessed for the average number of sporozoites per mosquito. Based on these results, the best batch is chosen for CHMI. Mosquitoes are then transferred from midi-cages to small CHMI-cages. Each step in this process is performed by an experienced technician, and checked and recorded on standardized forms by another technician according to standard operating procedures.

6.8 Drug accountability

The date, time of collection and person collecting both the Pb(PfCS@UIS4)- and *P. falciparum* NF54- infected mosquitoes is filled in on a standard table. This section will be signed by the responsible employee.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameters/endpoints

Phase 1

Primary endpoints

- Frequency and magnitude of adverse events in study groups.
- Presence of parasitemia after exposure to *Pb(PfCS@UIS4)* as assessed by thick smear.

Secondary endpoints:

- Immunogenicity of *Pb(PfCS@UIS4)* as assessed by ELISA and IFA.

Phase 2

Primary endpoints

- Frequency and magnitude of adverse events in study groups.
- Time to parasitemia after controlled human malaria infection with the wild-type *Plasmodium falciparum* NF54 strain, as detected by qPCR.

Secondary endpoints:

- Immunogenicity of *Pb(PfCS@UIS4)* as assessed by ELISA and IFA.

7.1.2 Exploratory parameters/endpoints

Phase 1

- To explore the composition and function of immune responses after exposure to Pb(PfCS@UIS4) including cytokine profile and Pb(PfCS@UIS4) induced antibodies and cellular (both adaptive and innate) immune responses

Phase 2

- To explore the composition and function of immune responses after exposure to Pb(PfCS@UIS4) including cytokine profile and Pb(PfCS@UIS4) induced antibodies and cellular (both adaptive and innate) immune responses
- To explore the changes in metabolomics profile of urine and serum/whole blood after CHMI.
- To determine cytokine responses, coagulation activation and inflammation parameters in non-immune individuals (controls) infected with *P. falciparum* during malaria liver phase.

7.2 Randomisation, blinding and treatment allocation

This is an open-label study. No randomisation or blinding will be performed.

7.3 Study procedures

7.3.1 Screening period: Screening, Inclusion and Baseline visits

Volunteers who wish to participate in the trial will be asked to complete an informed consent questionnaire. Their understanding of the trial will be tested after discussing the study with the investigator during informed consent, and prior to being asked to sign and date the consent form. Volunteers who fail to answer all questions correctly on their first attempt are allowed to re-take the questionnaire following further discussion with the investigator, and provided they subsequently answer all questions correctly, they may then complete the consent form and be screened for the trial.

Subjects who sign informed consent will undergo complete screening including a medical history, physical examination, vital signs, ECG, urine tests and laboratory evaluations (see sections 7.3.5-7.3.9). If, upon physical examination, vital signs or laboratory values are out of the normal range a repeat measurement may be obtained.

Subjects who meet the eligibility criteria will be invited back for enrollment into the study at the inclusion visit, which will occur 6 to 8 days prior to the planned immunization or challenge day (day 0). Some screening assessments, including physical examination, vital signs, urine tests and laboratory evaluations, will be repeated at this inclusion visit.

Following this screening period of up to 120 days, subjects who continue to meet the eligibility criteria (section 4.2 and 4.3) will present to the study site the day before the immunization or challenge infection for baseline assessments. Patient history will be taken and all adverse events that have occurred since screening will be noted. Only subjects who still meet the inclusion criteria will receive bites of infected mosquitoes. For each subject, the study start will be defined as the day of the inclusion visit.

7.3.2 Administration of *Pb(PfCS@UIS4)*

Phase 1

On the immunization day, all subjects will be exposed to the bites of five (group 1), twenty-five (group 2), or seventy-five (group 3) *Pb(PfCS@UIS4)* -infected mosquitoes in the Radboudumc in Nijmegen. Mosquito feeding will be allowed for 15 minutes. Volunteers will receive a local treatment (tripelennamine crème) for mosquito bites, if needed, and will be observed for 15 minutes after the feed. Directly after the feed, the mosquitoes will be

dissected (group 1 and group 2) or checked for blood-fed (group 3) by a technician of the mosquito unit according to standard operating procedures. Exposure will be repeated until five, twenty-five, or seventy-five infected mosquitoes have fed on each volunteer for group 1, 2 and 3, respectively.

As long as there are volunteers present in the mosquito unit, there will be supervision by one of the clinical investigators. Another clinical investigator will be on call, in case of emergency. Emergency aid kits will be present and readily available at any location, whenever there are volunteers present.

After the immunization, subjects will be observed closely according to an intensive out-patient follow-up schedule including frequent safety analyses (see section 7.3.14 for details). The study design is illustrated in more detail in the flowchart in section 7.3.14.

Subjects are required to reside locally within close proximity to the study site (within 1 hour from the Havenziekenhuis). In the cases where subjects are not local, subjects will be provided accommodation at a hotel nearby from the immunization day until day 12 post-immunization. For all subjects, during this period all relevant investigations will be carried out on an outpatient basis at the Havenziekenhuis, including frequent safety analyses (section 7.3.14 for details).

From the first day post-immunization up until the twentieth day post-immunization, assessments of parasitemia will be performed using thick smears on visit days. From day 21 until day 28 subjects will only be seen if they have relevant symptoms. Subjects will measure their body temperature twice daily, and the clinical investigators will contact all volunteers by telephone once daily to ask if there are any symptoms or complaints. Subjects of group 1 and 2, will visit the study site for a follow-up visit on day 1, 2 and 3 after treatment (TD+1, 2 & 3). Subjects of group 3 will visit on day 28, and no treatment will commence if considered safe. All subjects of group 1 and 2 will be seen for a final control visit on day 100 after immunization. Group 3 will subsequently start phase 2 when phase is considered safe. The study design is illustrated in more detail in the flowchart in section 7.3.14.

Thick smear assessment of parasitemia will be performed directly in volunteer samples.

As soon as a thick smear result is deemed positive for malaria parasites, the technician will inform the trial clinician. Treatment will be initiated after a single positive thick smear. If treatment has to be initiated, the trial clinician will immediately contact the volunteer, who will return to the clinic to receive Malarone® treatment. Preferably, the volunteer should return immediately or at least within 1 hour. If the volunteer is not reachable by phone, his/her contact person will be called and a search is started.

During the entire study period subjects will be instructed to call the trial physicians at any time if they experience symptoms. The trial physician can decide to initiate additional diagnostics or treatment at all times.
For unexpected laboratory abnormalities, the laboratory test can be repeated. If there is any ambiguity regarding the decision to include or exclude a volunteer, the clinical supervisor will discuss the case with the local safety monitor and make the final decision after that, if necessary with consultation of a specialist. If volunteers prove to be eligible, they will be invited to the next visit. If a volunteer is not fit to participate to undergo the immunization on the planned date, immunization may be delayed up to 10 days.
All subjects who are exposed to *Pb(PfCS@UIS4)* will be treated with Malarone®. Treatment after immunization will be based on the above mentioned criteria. Additionally, treatment can be initiated in any of the following situations:

1. By decision of study doctor or the safety monitor
2. In consultation with the cardiologist
3. On request of the volunteer
4. On day 28 post immunization, if the volunteer has remained thick smear negative
5. When LDH > 1000 U/l
6. When thrombocytes < 120 x 109/L

Phase 2

When phase 1 is terminated and is considered safe, the same volunteers of group 3 will be exposed three more times to bites of 75 mosquitoes infected with *Pb(PfCS@UIS4),*at four, four, and four to eight week interval. Three to four weeks after the last exposure all volunteers will undergo a controlled human malaria infection (CHMI) with 5 *P. falciparum* (NF54)-infected mosquitoes. Six infectivity control subjects will be recruited to receive 5 *P. falciparum* (NF54) infective mosquito bites per challenge infection.

Based on the first results of phase 2 an ad hoc decision will be made to include a potential booster immunization and/or subsequent second challenge infection with five *P. falciparum* (NF54)-infected mosquitoes. The booster immunization and/or subsequent second challenge infection will be initiated using the following criteria:

1. In case 2 to 4 volunteers are sterilely protected, all volunteers will receive a booster immunization followed by a second challenge infection, 3 months after the booster immunization.
2. In case ≥5 volunteers are sterilely protected, all non protected volunteers will receive a booster immunization followed by a second challenge infection 3 months later; all

protected volunteers will only receive a second challenge infection to assess durability of protection.

One day before the immunization days, all subjects will visit the clinical trial site for baseline assessments in the Havenziekenhuis in Rotterdam. On the immunization days, all subjects of group 3 will receive bites of seventy-five (group 3) *Pb(PfCS@UIS4)* -infected mosquitoes in the Radboudumc in Nijmegen. The same procedures will be performed as during the immunizations in phase 1.
After immunizations, subjects will be observed according to an out-patient follow-up schedule including safety analyses (see section 7.3.14 for details). The study design is illustrated in more detail in the flowchart in section 7.3.14.
Subjects are required to reside locally within close proximity to the study site (within 1 hour from the Havenziekenhuis). In cases where subjects are not local, subjects will be provided accommodation at a hotel nearby from the immunization day until day 8 post-immunization. For all subjects, during this period all relevant investigations will be carried out on an outpatient basis at the Havenziekenhuis, including frequent safety analyses (section 7.3.14 for details).

Subjects will visit the clinical trial site on day 1, and day 8 post-immunization. Safety analyses and assessment of parasitemia will be performed using thick smears on visit days. Thick smear assessment of parasitemia will be performed directly in volunteer samples. From day 1 until day 20 subjects will only be seen outside the normal visits if they have relevant symptoms. Subjects will measure their temperature twice daily, and the clinical investigators will contact all volunteers by telephone once every other day to ask if there are any symptoms or complaints.
During the entire study period subjects will be instructed to call the trial physicians at any time if they experience symptoms. The trial physician can decide to initiate additional diagnostics or treatment at all times.
For unexpected laboratory abnormalities, the laboratory test can be repeated. If there is any ambiguity regarding the decision to include or exclude a volunteer, the clinical supervisor will discuss the case with the local safety monitor and make the final decision after that, if necessary with consultation of a specialist. If volunteers prove to be eligible, they will be invited to the next visit. If a volunteer is not fit to participate to undergo the challenge infection or immunization on the planned date, immunization or challenge infection may be delayed up to 10 days.

All subjects who are exposed to *P. falciparum or Pb(PfCS@UIS4)* will be treated with Malarone®. Treatment after challenge infection will be based on the above mentioned criteria. Additionally, treatment can be initiated in any of the following situations:

1. By decision of study doctor or the safety monitor
2. In consultation with the cardiologist
3. On request of the volunteer
5. When LDH > 1000 U/l
6. When thrombocytes < 120 x 109/L

End of follow up will be 100 days after the last exposure to *Pb(PfCS@UIS4)* and 35 days post CHMI for the infectivity control subjects.

7.3.3 Controlled Human Malaria Infection

Three or four weeks after the last immunization of group 3, all subjects of group 3 and six infectivity controls will undergo a malaria challenge infection. Only subjects that met the inclusion criteria will undergo a malaria challenge infection. On the challenge day, all subjects will be exposed to the bites of five *P. falciparum* NF54 infected mosquitoes. Mosquito feeding will proceed for 10 minutes. Volunteers will receive a local treatment (tripelennamine crème) for mosquito bites and will be observed for 15 minutes after the feed. Directly after the feed, the mosquitoes will be dissected by a technician of the mosquito unit according to standard operating procedures. Exposure will be repeated until five infected mosquitoes have fed on each volunteer.

As long as there are volunteers present in the mosquito unit, there will be supervision by one of the clinical investigators. Another clinical investigator will be on call, in case of emergency. Emergency aid kits will be present and readily available at any location, whenever there are volunteers present.

After malaria challenge infection subjects will be observed closely according to an intensive out-patient follow-up schedule including frequent safety analyses (see section 7.3.14 for details). The study design is illustrated in more detail in the flowchart in section 7.3.14.

For all subjects, during this period all relevant investigations will be carried out on an outpatient basis at the Havenziekenhuis, including frequent safety analyses (section 7.3.14 for details).

From the sixth day up until the twenty-first day post-CHMI, assessments of parasite densities using qPCR will be performed once daily. From day 22 until day 28 subjects will only be seen if they have relevant symptoms. Groups 4 and 5 will have two additional visits on day 4 and 5 post-CHMI for explorative add-on studies. Subjects will measure their temperature twice daily, and the clinical investigators will contact all volunteers by telephone once daily to ask if

there are any symptoms or complaints. Subjects will visit the study site for a follow-up visit on Days 1, 2 and 3 after treatment (TD+1, 2 & 3). All subjects will be seen for a final control visit on day 35 after CHMI. The study design is illustrated in more detail in the flowchart in section 7.3.14.

qPCR assessment of parasite densities will be performed directly in volunteer samples.

As soon as a qPCR or thick smear result is deemed positive for malaria parasites, the technician will inform the trial clinician. Treatment will be initiated after a single positive qPCR. If treatment has to be initiated, the trial clinician will immediately contact the volunteer, who will return to the clinic to receive Malarone® treatment. Preferably, the volunteer should return immediately or at least within 1 hour. If the volunteer is not reachable by phone, his/her contact person will be called and a search is started.

During the entire study period subjects will be instructed to call the trial physicians at any time if they experience symptoms. The trial physician can decide to initiate additional diagnostics or treatment at all times.

For unexpected laboratory abnormalities, the laboratory test will be repeated. If there is any ambiguity regarding the decision to include or exclude a volunteer, the clinical supervisor will discuss the case with the local safety monitor and make the final decision after that, if necessary with consultation of a specialist. If volunteers prove to be eligible, they will be invited to the next visit. If a volunteer is not fit to participate to undergo the challenge infection on the planned date, challenge infection may be delayed up to 10 days.

All subjects who are exposed to *P. falciparum* will be treated with Malarone®. Treatment after challenge infection will be based on the above mentioned criteria. Additionally, treatment can be initiated in any of the following situations:

1. By decision of study doctor or the safety monitor
2. In consultation with the cardiologist
3. On request of the volunteer
4. On day 28 post CHMI, if the volunteer has remained qPCR-negative
5. When LDH > 1000 U/l
6. When thrombocytes < 120 x 109/L

A second malaria challenge infection might be initiated as described in section 7.3.2, and all procedures will be similar. Six new infectivity controls will be included.

7.3.4 Treatment with Malarone®

All volunteers will be treated with Malarone® (atovaquone/proguanil) based on the predetermined criteria mentioned above. The treatment will consist of the drug Malarone®. Dosing will be as follows: once daily 4 tablets of 250/100mg, during three days, according to

Dutch SWAB guidelines. This drug has been chosen because of its fast clinical response and the few side-effects. Furthermore, it has not been reported to have any cardiac side-effect. During treatment, complaints of malaria infection will be treated symptomatically. In addition to specific treatment with Malarone®, symptomatic treatment will be administered at the discretion of the study physician.
During and one day after Malarone® treatment, qPCR is performed directly in collected blood samples. If qPCR remains positive after Malarone® treatment (usually the result of parasite debris remaining in the bloodstream) a thick blood smear will be performed to confirm the absence of intact malaria parasites.
Volunteers will not be admitted to the hospital during this study, unless the study doctors or the safety monitor deems it necessary, or on request of the volunteer.

7.3.5 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.

7.3.6 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, with back supported and both feet placed on the floor, using an automated 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.

7.3.7 Patient-reported outcomes (study diary)

At the inclusion visit, subjects will be issued symptom diaries and thermometers. They will be asked to record all symptoms and medication use daily from the day of inclusion until end of study. The subject study diary will be reviewed at each study visit and used as a basis for discussion of possible adverse events or medication use. If the occurrence of an adverse event or use of medication is confirmed by the study physician, it is recorded in the subject's study file.

Subjects will also be asked to measure their temperature orally every morning from the day of the immunization until day 28 (phase 1) or day 8 (phase 2), or after the malaria challenge infection until the third day of Malarone® (atovaquone/proguanil) treatment, and record this temperature in their study diaries.

At the end of the study, the diary will be collected and kept as source data with the subject's study file.

7.3.8 Electrocardiogram

A standard 12 lead ECG will be performed at screening. 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 min rest in the supine position. Calibration should be performed per the local site/requirements. Each ECG tracing should be labelled 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 should also be recorded in the CRF.

7.3.9 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 (section 7.3.14) shows when blood will be drawn. Hemoglobin, hematocrit, red blood cell count, white blood cell count with differential (e.g. neutrophils, basophils, eosinophils, monocytes, lymphocytes) and platelet count will be measured at regular time points during the study.

Alkaline phosphatase, total bilirubin, creatinine, γGT, LDH, potassium, AST, ALT, sodium, highly sensitive troponin T and urea will be measured at regular time points during the study. Glucose, triglycerides and cholesterol will be measured only at screening.

In the event that an asymptomatic individual has evidence of an elevated troponin level, a second sample may be obtained to discern whether the result could represent a false positive (0.4% of tests). At the screening visit plasma samples will be tested for HIV, hepatitis

B and hepatitis C. Additionally, serum will be tested for antibodies to malaria parasites by ELISA.

A midstream urine sample (approx. 30 ml) will be obtained at screening, inclusion and, for female subjects only, at baseline before every immunization and/or challenge infection. In this sample the presence of amphetamines and cocaine will be assessed; the sample taken at inclusion will also be tested for the presence of cannabis. Additionally, for female participants a commercially available hCG urine test will be used to test for pregnancy at screening and baseline before every immunization and/or challenge infection.

In the case where a laboratory assessment is outside the reference range for the laboratory at screening and/or inclusion, 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.

7.3.10 Analysis of asexual parasite densities after challenge infection

qPCR for assessment of parasite densities will be performed directly in volunteer samples, as discussed previously (see flowchart 7.3.14)

qPCR is performed according to a standard procedure as previously described (Hermsen et al., 2001) with small adjustments. In short, qPCR will be performed for 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 volunteer samples, as discussed previously and if deemed necessary by the clinical investigator (see section 8.3.2), according to a standard operating procedure which is based on an internationally harmonized protocol for thick smears in CHMIs (Moorthy et al., 1998 and WHO, 2010). Per slide, the number of fields correlating to 0.5 µl of blood will be read. Slides are considered positive if they contain 2 or more parasites in these fields.

7.3.11 Immunological assays

Blood samples will be taken for isolation of peripheral blood mononuclear cells (PBMCs) and plasma and serum (see flowchart 7.3.14).

PBMCs and both plasma and serum will be frozen and can then be used by the Radboudumc or its collaborators for exploratory immunological assays to further analyze immunity induced by *Pb(PfCS@UIS4)*, and to analyze the phenotype or functionality of the immune response during and after malaria infection.

Humoral assessment will include antibody assays by immunofluorescence, and ELISA's for specific PfCS- and Pb-proteins. In addition, antibody functionality will be tested in a range of *In vitro* assays, such as inhibition of sporozoites invasion or maturation assays in primary human hepatocytes or hepatoma cell lines.

Cellular assessment of parasite-specific (subset)T-cell response will be conducted by multi-parameter flow cytometry and ELISpot assay following Pf or Pb stimulation *In vitro.*
In order to assess (antigen specific) T cell responses, the HLA-type of volunteers may be determined.

7.3.12 Cytokines

Cytokine levels will be measured to assess in vivo inflammatory responses after immunization with *Pb(PfCS@UIS4).*
In phase 1 cytokines such as, IL-1β, TNF-α, IL-6, IL-8, IL-10, IL-17, and IFNy will be measured by ELISA or Luminex multiplex assay, on day 1 to 10 after administration of *Pb(PfCS@UIS4).*

7.3.13 Other

During the *P. falciparum* challenges, sampling of material such as urine, serum, citrated and EDTA whole blood will be collected on a daily basis, for add on studies on pathogenesis of early malaria. In these samples the vasopressin response, haemostasis and cholesterol metabolism will be studied by determination of several parameters with standard clinical laboratorial techniques and ELISAs. Furthermore, the deformability of erythrocyte will be measured by a Rheo scan.

Investigation of early malaria pathogenesis and the host immune responses during liver phase has never comprehensively been studied in humans. Recently, leukocyte counts and leukocyte differentiation were proven to change during malaria liver phase [35]. To investigate the changes in proteins and metabolites in blood metabolomics during the liver phase in more detail, volunteers of group 4 and 5 will be exposed to two extra visits with blood sampling (day 4 and 5 post-CHMI). The following parameters will be determined: cytokine levels (e.g. IL-6; IL-10, IL-12, TGF-β, IFN-γ and TNF-α as measured by ELISA or Luminex multiplex assay), and coagulation factors (e.g. prothrombin fragment 1.2, D-dimer, platelet count, von Willebrand factor, von Willebrand factor pro-peptide will be measured by ELISA). In addition, standard hematology- and biochemistry tests will be assessed on these

time-points. The samples that will be taken are: citrate vial 2.7 ml; CTAD vial 2.7 ml; EDTA vial 4.0; serum vial 5.0 ml and urine sample 10 ml. Results of tests will only be analyzed after day 35 of the challenge to avoid bias in clinical evaluation or approach.

7.3.14 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. The diaries, produced by the study volunteers are also considered source data. They will be kept as source documents together with the investigator's notes.

7.3.15 Flowchart trial procedures: Phase 1

	Screening	**Inclusion**	**Baseline**	**Administration Pb(PfCS@UIS4)**			**EOS**	
Visit Number	V1	V2	V3	V4	V5-14	V15-19	V20-23	V24	V25
Trial timeline	-120 to -11	-8 to -6	-1	0	1-10	12-20	28-31 (TD+1-3)	35	100
Number of visits	1x	1x	1x	1x	10x	5x	4x	1x	1x
Informed consent	X								
Eligibility criteria	X		X						
Demographic data, Medical history	X								
Physical examination and vital signs	X	X			X[8]	X[8]	X[8]	X[8]	X[8]
ECG	X								
Temperature	X	X	X		X	X	X	X	X
Immunization by mosquito bites				X					
Collecting (serious) adverse events				as necessary[9]					
Treatment Day(TD; Malarone®)					X[10]	X[10]	X[10]		
Haematology tests[1]	X	X	X		X	X	X	X	X
Biochemistry tests[2]	X	X			X[11]		X[11]	X	X
HsTropT + LDH			X		X	X	X		
Serology[3]	X	X							
Pregnancy and toxicology urine test[4]	X	X	X						
Parasitology[5] (thicksmear)					X	X	X	X	X
Parasitology[5] (qPCR)			X						
Exploratory immunology			X		X	X	X	X	X
Safety report[6]						X			X
Total blood volume collect (ml)[7]	15	11.5	73	0		163.5		9	49

[1] Hemoglobin, hematocrit, platelets, red blood cell count, white blood cell count + differentiation (differentiation only on inclusion and day 100)

[2] Creatinine, urea, sodium, potassium, bilirubin, AF, γGT, AST, ALT, LDH and hs-Trop-T. Additional at screening: cholesterol, triglyceride + glucose

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

[4] Toxicology urine tests at screening and inclusion visits only. Pregnancy test for female subjects at screening and baseline visits (before every

immunization/challenge)

[5] Thicksmear will be taken from same sample as qPCR.18S qPCR for blood stage *P. berghei* will only be assessed on samples retrospectively.

[6] A safety report will be compiled upon all subjects of group 1 and 2 having completed day 12 after immunization, day 28 for group 3, and at the end of the study,

[7] Total blood volume: 321ml

[8] On indication.

[9] Only SAE's that are possibly or probably related to trial will be collected prior to inclusion.

[10] If positive thicksmear or on day 28 post-immunization

[11] Biochemistry tests will be performed on day 2, day 4, day 12 post immunization and TD+2.

Flow chart trial procedures: Phase 2

	Inclusion	**Immunization 2**			**Immunization 3**				**Immunization 4**				**CHMI**				**EOS**	
Visit Number	V21	V22	V23	V24	V25	V26	V27	V28	V29	V30	V31	V32	V33	V34	V35-V50	V51-V54	V55	V56
Trial timeline	-29	30	31	38	57	58	59	66	113	114	115	122	134	135	141-156	163-166	170	214
Number of visits	1x	1x	1x	1x	1x	1x	1x	1x	1x	1x	1x	1x	1x	1x	16x[14]	4x	1x	1x
Informed consent																		
Eligibility criteria	X																	
Demographic data, Medical history																		
Physical examination and vital signs	X[9]		X[9]	X[9]	X[9]		X[9]	X[9]	X[9]		X[9]	X[9]	X[9]		X[9]	X[9]	X[9]	X[9]
ECG																		
Temperature	X		X	X	X		X	X	X		X	X	X		X	X		
Immunization with *Pb(PfCS@UIS4)*		X				X				X								
Challenge with *P. falciparum*														X				
Collecting (serious) adverse events									as necessary[10]									
Treatment Day(TD; Malarone®)			X[11]	X[11]	X[11]		X[11]	X[11]	X[11]		X[11]	X[11]			X[12]	X[12]		
Haematology tests[1]	X		X	X	X		X	X	X		X	X	X		X	X	X	X
Biochemistry tests[2]	X			X				X				X	X			X[13]	X	X
HsTropT + LDH			X		X		X		X		X		X		X			
Serology[3]																		
Pregnancy and toxicology urine test[4]	X				X				X				X					
Parasitology[5] (thicksmear)			X	X			X	X			X	X						
Parasitology[5] (qPCR Pb)	X		X	X	X		X	X	X		X	X					X	X
Parasitology[6] (qPCR Pf)													X		X	X	X	X
Exploratory immunology	X				X				X				X		X	X		X
Safety report[7]					X				X				X					X
Total blood volume collect (ml)[8]	49	0	6.5	6.5	49	0	6.5	6.5	49	0	6.5	6.5	73	0		330.5	58.9	58.9

[1] Hemoglobin, hematocrit, platelets, red blood cell count, white blood cell count + differentiation (differentiation only on inclusion and day 100)

[2] Creatinine, urea, sodium, potassium, bilirubin, AF, γGT, AST, ALT, LDH and hs-Trop-T. Additional at screening: cholesterol, triglyceride + glucose

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

[4] Pregnancy test for female subjects at baseline visits (before every immunization/challenge). Toxicology urine tests only at baseline prior challenge infection

[5] Thicksmear will be taken from same sample as qPCR.18S qPCR for blood stage *P. berghei* will only be assessed on samples retrospectively.

[6] If positive qPCR for *P. falciparum.* Thicksmear only on indication (sample taken from qPCR sample).

[7] A safety report will be compiled upon all subjects per group having completed day 28 after immunizations (with exception of day 21 for the 4th immunization) and at the end of the study.

[8] Total blood volume: 707.3ml

[9] On indication.

[10] Only SAE's that are possibly or probably related to trial will be collected prior to inclusion.

[11] If positive thicksmear

[12] If positive qPCR for bloodstage *P. falciparum*

[13] Biochemistry tests on C+6, TD TD+2

[14] Two additional visits for group 4 are scheduled (visit 34a and 34b) on day 4 and 5 after infection. Not included during these extra visits: physical examination and vital signs, qPCR Pf, HS Trop T, LDH.

Flow chart trial procedures: Phase 2 – adaptive design

	Immunization 5				**CHMI 2**				**EOS**
Visit Number	V57	V58	V59	V60	V61	V62	V63-78	V79-V82	V83
Trial timeline	225	226	227	234	309	310	316-331	338-341	345
Number of visits	1x	1x	1x	1x	1x	1x	16x[14]	4x	1x
Informed consent									
Eligibility criteria	X								
Demographic data, Medical history									
Physical examination and vital signs	X[9]		X[9]	X[9]	X[9]		X[9]	X[9]	X[9]
ECG									
Temperature	X		X	X	X		X	X	
Immunization with *Pb(PfCS@UIS4)*		X							
Challenge with *P. falciparum*						X			
Collecting (serious) adverse events					as necessary[10]				
Treatment Day(TD; Malarone®)	X[11]		X[11]	X[11]			X[12]	X[12]	
Haematology tests[1]	X		X	X	X		X	X	X
Biochemistry tests[2] (				X	X			X[13]	X
HsTropT + LDH	X		X		X		X		
Serology[3]									
Pregnancy and toxicology urine test[4]	X				X				
Parasitology[5] (thicksmear)			X	X					
Parasitology[5] (qPCR Pb)	X		X	X					X
Parasitology[6] (qPCR Pf)					X		X	X	X
Exploratory immunology	X			X	X		X	X	
Safety report[7]	X				X				
Total blood volume collect[8]	49	0	6.5	6.5	57	0	330.5		59

[1] Hemoglobin, hematocrit, platelets, red blood cell count, white blood cell count + differentiation (differentiation only on inclusion and day 100)

[2] Creatinine, urea, sodium, potassium, bilirubin, AF, γGT, AST, ALT, LDH and hs-Trop-T. Additional at screening: cholesterol, triglyceride + glucose

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

[4 4] Pregnancy test for female subjects at baseline visits (before every immunization/challenge). Toxicology urine tests only at baseline prior challenge infection

[5] Thicksmear will be taken from same sample as qPCR.18S qPCR for blood stage *P. berghei* will only be assessed on samples retrospectively.

[6] If positive qPCR for *P. falciparum.* Thicksmear only on indication (sample taken from qPCR sample).

[7] A safety report will be compiled upon all subjects per group having completed day 28 after immunizations and at the end of the study.

[8] Total blood volume: 508.5ml

[9] On indication.

[10] Only SAE's that are possibly or probably related to trial will be collected prior to inclusion.

[11] If positive thicksmear

[11] If positive qPCR for bloodstage *P. falciparum*

[13] Biochemistry tests on C+6, TD, and TD+2

[14] Two additional visits for group 5 are scheduled (visit 62a and 62b) on day 4 and 5 after infection. Not included during these extra visits: physical examination and vital signs, qPCR Pf, HS Trop T, LDH.

7.4 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.

Volunteers 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.*
- Pregnancy.
- Withdrawal of informed consent by volunteer.
- 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.
- Volunteer non-compliance with study requirements.
- Any other protocol deviation that results in a significant risk to the subject's safety.

If withdrawal of a subject 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, and follow the subject for cardiac manifestations of disease. 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, etc.

7.5 Replacement of individual subjects after withdrawal

If a subject withdraws before or on inclusion day he/she will be replaced with an alternate volunteer who passed screening, if possible.

7.6 Follow-up of subjects withdrawn from treatment

In the event that a volunteer 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 volunteers who have been exposed to the bites of infectious mosquitoes are required to take a curative regimen of Malarone® (or alternative effective anti-malarial treatment should Malarone® be contra-indicated, at the discretion of the clinical investigator).

7.7 Procedure in case of pregnancy

Pregnancy itself is not considered an adverse event, but any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an adverse event or serious adverse event.

The investigator will record the pregnancy information on a pregnancy notification form (as described in standard operating procedures) and submit it to the local safety monitor, and sponsor within 24 hours of learning of a subject’s pregnancy. The subject will be followed to determine the outcome of pregnancy. At the end of the pregnancy, information on the status of the mother and child will be forwarded to the sponsor. Follow-up will generally be no longer than six to eight weeks following delivery date.

Volunteers who become pregnant during the study will not receive additional immunizations or challenges, but may continue other study procedures at the discretion of the investigator.

All female volunteers should refrain from getting pregnant until the last study visit.

7.8 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).
- On advice of the clinical investigator.
- On advice of the CCMO.
- If the predefined holding criteria are met (section 8.4.4)

The safety monitor, SMC, CCMO or investigators may decide to put the study on hold based on adverse events, pending discussion with the Sponsor / SMC / CCMO / safety monitor / investigators. Following discussion, it may be decided to terminate the study. Safety reporting procedures are described in section 8.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the CCMO without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subject's health. The investigator will take care that all subjects are kept informed.
PATH REC (PATH Research Ethics Committee) and WIRB will also be notified of any decisions to prematurely suspend or terminate the study.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product or study intervention. AEs may include events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen). All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

Abnormal laboratory findings (e.g., clinical chemistry, haematology, urinalysis) 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 volunteer 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.

Any hs-troponin value above 14ng/L will be recorded as adverse event (grade 1). For any hs-troponin value above 60ng/L the cardiologist will be consulted, an electrocardiogram will be made (grade 2) and the local safety monitor will be informed within 24 hours. The limit of detection of the hs-troponin T assay is 3 ng/L. The 99th percentile cut-off point is 0.014 ng/mL (14ng/L). Further procedures, such as cardiac MRI's will be performed on advice of the cardiologist. Any hs-troponin above 100 ng/L will be considered a grade 3 (severe) adverse event.

8.2.2 Serious adverse events (SAEs)

A serious adverse event (SAE) 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.

To ensure no confusion or misunderstanding of the difference between the terms 'serious' and 'severe', which are not synonymous, the following note of clarification is provided:

The term 'severe' is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as 'serious', which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. (Example: a grade 3 AE is not necessarily a SAE.)

The principle investigator of each participating centre is responsible for reporting of the Serious Adverse Event to the sponsor within 24 hours of occurrence of the event. The investigator (on behalf of the sponsor) will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the

sponsor has first knowledge of the serious adverse events. The Sponsor will notify the SMC of the SAE within 24 hours of being notified of the SAE.

SAEs that result in death or are life threatening should be reported in an expedited fashion. The expedited reporting to the accredited METC will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is in the form of a preliminary report, a complete report will be provided by the sponsor no later than 15 days after first knowledge of the SAE.

8.2.3 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).

The sponsor will report expedited all SUSARs through the web portal *ToetsingOnline* to the CCMO. The expedited reporting of SUSARs through the web portal *ToetsingOnline* 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 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 PATH REC and/or WIRB in accordance with the reporting requirements of each.

8.3 Follow-up of (serious) adverse events

8.3.1 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, a new description of the AE will be added. Interrupted AEs are registered as one AE if the interruption is <24 hours.

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.

After every immunization the following signs and symptoms will be solicited at any visit after immunization in phase 1 or at any visit in phase 2 until CHMI:
Fever (by examination), rash, urticaria, pruritis, edema, headache, fatigue, malaise, rigors, myalgia, arthralgia, dizziness, sweats, cough, nausea, vomiting, abdominal pain, diarrhoea,

The following signs and symptoms local to the site of mosquito bites will be solicited after every immunization: Tenderness, induration (assessed by palpation), bruising/extravasated blood, erythema, swelling (assessed by direct or lateral visualization), and pain, pruritis (subjective symptoms).

The following signs and symptoms will be solicited after CHMI in stage B:
Fever (by examination), rash, urticarial, pruritis, edema, headache, fatigue, malaise, rigors, myalgia, arthralgia, dizziness, sweats, cough, nausea, vomiting, abdominal pain, diarrhoea, chest pain, palpitations, shortness of breath.

8.3.2 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:

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.
Not related	An 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.

8.3.3 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 volunteer 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 within the Netherlands, defined as the last patient visit.

8.4 Local Safety Monitor (LSM) and Safety Monitoring Committee (SMC)

8.4.1 Local safety monitor

For this study, a local safety monitor will appointed, who is based in the Havenziekenhuis and will be involved in the review of severe and serious adverse events and volunteer 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, funding partner, and the investigators.

8.4.2 Safety Monitoring Committee (SMC)

An independent Safety Monitoring Committee (SMC) 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 on specific days as detailed in section 7.3.15.

The advice(s) of the SMC will only be sent to the sponsor of the study and the funding partner, PATH. Should the sponsor decide not to fully implement the advice of the SMC, the sponsor will send the advice to the CCMO, including a note to substantiate why (part of) the advice of the SMC will not be followed.

8.4.3 Review of safety data by the safety monitor and SMC

A safety report including a list of all reported adverse events and any safety laboratory values outside the normal range will be prepared specific days as detailed in section 7.3.15.

These reports will be prepared by a clinical investigator and sent to the safety monitor, all clinical investigators involved and the funding partner, PATH. 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 at the end of the study. Responsibilities of the SMC are described in the SMC Charter. The advice(s) of the SMC will only be communicated to the CCMO when the sponsor does not follow this. With this notification a statement will be included indicating whether the advice will be followed.

Any highly sensitive troponin T value greater than 60 ng/l will be reported to the safety monitor within 24 hours. Any safety laboratory values that lead to immediate malaria treatment will be reported to the safety monitor within 24 hours.

8.4.4 Safety holding 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 SMC.
- On advice of the CCMO.
- When individual or group holding criteria are met (see section 8.4.4.1 and 8.4.4.2).

In phase 1, the trial will proceed from group 1 to group 2 to group 3 as long as the holding criteria are not met. At any time if holding criteria are met, an ad hoc meeting of the SMC will be called.

The safety monitor, CCMO, or investigators may decide to put the study on hold based on adverse events, pending discussion with the safety monitor, CCMO, 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 SMC will be responsible for granting approval for proceeding from phase 1 to phase 2, and before the challenge infection. If the CCMO has recommended safety hold, re-initiation of the study will require CCMO concurrence. The CCMO, PATH REC and WIRB will be informed of a safety hold by the sponsor. Following discussion, it may be decided to terminate the study.

8.4.4.1 Individual holding rules

The following Individual Holding Rules will apply to each subject. The PI will monitor the individual holding rules, and will make recommendations regarding the continuation of immunizations in a subject. If the individual holding rules are met, no further immunizations will be administered to that subject until the investigators have conferred with the local safety monitor and a full written report has been submitted to PATH.

- Local Reactions: The investigator, at his or her discretion, can hold an individual's immunization based on a local reaction.
- Clinical systemic adverse events (AEs): Immunization possibly or probably related unsolicited Grade 3 AEs beginning within 28 days following immunization and persisting at Grade 3 for greater than 48 hours.

- Laboratory systemic AEs: Immunization possibly or probably related Grade 3 abnormalities beginning within 7 days following immunization and persisting at Grade 3 for greater than 48 hours.
- Systemic AEs: Acute systemic allergic reaction or anaphylactic shock following the administration of *Pb(PfCS@UIS4).*
- Positive urine pregnancy test.
- Serious adverse event (SAE) determined to be possibly or probably related to the administration of *Pb(PfCS@UIS4).*
- The occurrence of a Pb(PfCS@UIS4) blood stage infection (detected by thick smear) in one of the volunteers in phase 1 or 2.

If a subject has an acute illness (moderate or severe illness with or without fever) or a fever (oral temperature greater than or equal to 38.0°C) at the scheduled time of administration of *Pb(PfCS@UIS4)*, the subject will not receive the immunization at that time. The immunization may be administered to that subject at a later date within the time window specified in the protocol or the subject may be withdrawn from the study at the discretion of the investigator.

After an individual holding rule is activated, a thorough review by the PI, local safety monitor, and sponsor will occur. Immunization of the subject may resume only if all parties agree it is safe to resume immunization.

All immunized subjects will be followed for safety until resolution or stabilization (if determined to be chronic sequelae) of their AEs.

8.4.4.2 Group holding rules

The following holding rules apply to each group of subjects. Solicited and unsolicited adverse events and safety laboratory data will be recorded and tracked by the PI after each immunization. The local safety monitor will review the AEs (local, clinical systemic, and laboratory systemic) and will make recommendations regarding the continuation of the study for each group. If the group holding rules are met, no further immunizations will be administered in any group until the investigators have conferred with the local safety monitor and a full written report has been submitted to PATH.

- Local Reactions: Upon investigator discretion.
- Clinical systemic AEs: immunization possibly or probably related unsolicited Grade 3 AEs in the same organ system beginning within 28 days following immunization and

persisting at Grade 3 for greater than 48 hours in 2 or more subjects within group 1 or within group 2, or 4 or more subjects within group 3 following each immunization.

- Laboratory systemic AEs: immunization possibly or probably related Grade 3 abnormalities beginning within 7 days following immunization and persisting at Grade 3 for greater than 48 hours in 2 or more subjects within group 1 or within group 2, or 4 or more subjects within group 3 following each immunization.
- SAE determined to be possibly or probably related to the immunization.
- The occurrence of a Pb(PfCS@UIS4) blood stage infection (detected by thick smear) in one of the volunteers in phase 1 or 2.

After a group holding rule is activated, a thorough review by the PI, local safety monitor, and sponsor will occur. Immunization of the subjects within the affected group and the other immunization groups may resume only if the local safety monitor, the PI, and the sponsor agree it is safe to resume immunizations and the following considerations are discussed:

- Relationship of the AE or SAE to the immunization.
- Relationship of the AE or SAE to the immunization dose (ie, only associated with high dose group).
- If appropriate, additional screening or laboratory testing is provided to other subjects to identify subjects who may develop similar symptoms.
- If any study related SAE is not listed on the current informed consent form (ICF), the PI will revise the ICF and subjects will be asked to provide consent on the new ICF.

All immunized subjects will be followed for safety until resolution or stabilization (if determined to be chronic sequelae) of their AEs.

8.4.5 Deferral of study procedures for individuals

Deferral of trial procedures for individual subjects could be considered when health problems based on history and clinical examination (physical examination and laboratory tests) are present. Details regarding these problems will be assessed in concurrence with appropriate specialists and if necessary with the local safety monitor. Subsequently, a volunteer-specific decision regarding deferral or continuation of study procedures for this particular individual will be made e.g. a subject with a positive pregnancy test before immunization will obviously be excluded from the study, while a subject suffering from a clinically significant common cold will be able to continue study procedures possibly in the next group once symptoms diminish. If a volunteer is not fit to participate to undergo the challenge infection of immunization on the planned date, challenge infection may be delayed up to 10 days.

9 STATISTICAL ANALYSIS

9.1 Primary study parameter(s)

All volunteers exposed to *Pb(PfCS@UIS4)* will be included in the intention-to-treat analysis.
The primary endpoint is e*valuated by t*abulating all adverse events for each volunteer. Adverse events will be analysed by calculating the proportion of volunteers in each group who reported mild, moderate or severe adverse events. The frequency of signs and symptoms will be compared between groups with the Fisher's exact test.
Presence of parasitemia after exposure to *Pb(PfCS@UIS4)* will be assessed by thick smear. After challenge infection, the time to *P. falciparum* positivity (positive qPCR >100 *Pf*/ml) will be compared between groups with the Mann-Whitney U test.

9.2 Exploratory study parameters

In the exploratory immunological analyses we will assess differences by comparing mean values between the groups using either the Fisher's exact test or Wilcoxon signed rank test, paired if pre-exposure values are compared with post-exposure values, unpaired if comparisons are made between groups. For discrete variables (e.g. the number of positive assays), non-parametric tests or, depending on the distribution, analyses based on count data such as Poisson regression are used.

10 ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study will be conducted in accordance with the latest Fortaleza revision of the Declaration of Helsinki (2013), the Medical Research Involving Human Subjects Act (WMO), the ICH Good Clinical Practice, and local regulatory requirements.

The investigators are responsible for obtaining all relevant Ethics Committee (EC) / Institutional Review Board (IRB) approvals, including WIRB approval, of the protocol and any subsequent amendments in compliance with local law before the start of the study.

10.2 Recruitment and consent

As soon as the study is approved by the CCMO and WIRB, healthy volunteers will be recruited to participate in the study. Advertisements will be placed in prominent places on university campuses and other public places as well as on the intranet of the Erasmus University. Furthermore, a Facebook page (link: http://www.facebook.com/malariavaccin) showing the advertisement text will be designed to inform people about the trial. This brief advertisement will indicate a telephone number to call and an e-mail address for contact to request further information. It will furthermore indicate a website which contains a link to a short online form. This short online form requests the interested volunteer to provide their contact details and answer some initial general health and medical questions. When seemingly suitable volunteers contact investigators via e-mail, telephone or the online form, they will be invited to an information meeting during which the study will be explained to them by the study investigator. Directly after the meeting they will be provided with documents to review at home (the information sheet, the informed consent form, the application form, and the insurance text). 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 volunteer will be given sufficient time to consider participation.

Volunteers 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, CCMO and WIRB-approved informed consent. Informed consent must be obtained after the informed consent questionnaire and before conducting any study-specific procedures (i.e. any 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 informed consent questionnaire and health questionnaire answers will be discussed, and inclusion and exclusion criteria will be checked. Also, a letter for the general practitioner will be signed and sent after screening. Again, the investigator will answer all questions the volunteer has. The

investigator will emphasize that participation in the study is entirely voluntary and that the subject may withdraw at any time, without any obligation to declare their reason for withdrawal. However, if the volunteer has undergone exposure to *Pb(PfCS@UIS4)* or CHMI and not completed a course of appropriate antimalarial therapy then the volunteer will need to maintain contact with the investigators for monitoring and treatment.
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 volunteers. Volunteers 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.

10.3 Benefits and risks assessment, group relatedness

In order to obtain information on the capacity of potential immunization strategies to induce protection, immunization strategies will need to be tested in human subjects. Of course, the compelling need for a malaria vaccine needs to be balanced with the potential risks and discomforts for the volunteers. Explorative studies of potential pre-erythrocytic malaria vaccine candidates are a potentially powerful tool in the difficult decision making process of continuing investigation into vaccine candidates. This safety trial will be pivotal to the development of *Pb(PfCS@UIS4)* and will be used as a go/no-go criterion for further pursuit of *Pb(PfCS@UIS4)* as a potential vaccine candidate.
There are no direct benefits to participation in the trial for volunteers. Despite their participation, volunteers will be advised to take regular malaria chemoprophylaxis when travelling to malaria endemic areas in the future.
Risks for volunteers of phase 1 are related to exposure to a *i)* potential break-through blood stage infection after exposure to *Pb(PfCS@UIS4)* sporozoites, *ii)* the side effects of Malarone® (atovaquone/proguanil) and *iii)* side effects related to exposure to multiple mosquito bites.
The volunteers of phase 2 will be exposed to the same risks as phase 1. However, the risk of blood stage infection after administration of *Pb(PfCS@UIS4)* is considered lower, since an absence of break-through infections in phase 1 is a criterion for proceeding to phase 2. Instead, volunteers of phase 2 have the additional risk *iv)* to develop blood-stage malaria after wild type *P.falciparum* NF54 infection.

i) Risks related to administration of Pb(PfCS@UIS4)

Based on preclinical assessment the occurrence of ‘break-through’ blood infections by *Pb(PfCS@UIS4)* is highly unlikely (please refer to D1 Investigator’s Brochure for a detailed

description of the pre-clinical studies. To ensure prompt treatment of a possible blood stage infection and adequate counselling and support, volunteers will be subjected to an intense clinical monitoring schedule with frequent site visits and blood examinations, comparable to protocols used in CHMI studies.

Volunteers with breakthrough blood infections of *Pb(PfCS@UIS4)* will be treated with Malarone® (atovaquone/proguanil). *In vitro* and *in vivo* drug tests show that *Pb(PfCS@UIS4)* is sensitive to drugs such as atovaquone/proguanil and artemether/lumefantrine (please also refer to D1 Investigator's Brochure). It is thus expected that a 100% cure rate will be met with these drugs, similar to blood infections of *P. falciparum* NF54 in CHMI studies.

ii) Risks related to exposure to atovaquone/proguanil treatment

Side effects associated with atovaquone/proguanil treatment are mainly gastrointestinal such as nausea, with vomiting, abdominal pain and diarrhea, and only present in a minority of cases. Headache, dizziness, anorexia and allergic reactions have been described. However, previous experience with Malarone® (atovaquone/proguanil) treatment in CHMI has demonstrated that side-effects are generally mild-to-moderate and limited to the days of treatment only (3 days). In case of unacceptable side effects, artemether/lumefantrine can be used as alternative treatments.

iii) Side effects related to exposure to multiple mosquito bites

The exposure of test subjects to mosquito bites has been shown to induce local adverse events such as itching, redness and swelling, the severity of which varies among test subjects. Topical tripelennamine and topical steroids such as 1% hydrocortisone cream and for more severe reactions, clobetasol 0.05% cream will be available in the clinical trials center for distribution to the volunteers. Additional medications available to volunteers by the clinical investigators will be oral cetirizine or other anti-histamines. Volunteers with exuberant reactions to mosquito bites may receive cetirizine prophylactically before immunization to abrogate skin reactions such as redness, swelling and itching. Systemic adverse events elicited by mosquito bites have not been found previously. It is not expected that bites of mosquitoes infected with *Pb(PfCS@UIS4)* will alter these events. In addition to local events, the administration of irradiated sporozoites by mosquito bite has led to the occurrence of mild headache and/or malaise in a limited number of volunteers.

iv) Risks related to exposure to blood stage NF54 parasites (phase 2 only)

There is a broad experience with the use of NF54 to induce blood stage malaria infection during CHMI the Radboudumc, ErasmusMC and Havenziekenhuis.

The occurrence of cardiac events in volunteers after blood stage malaria infection during CHMI trials in the Radboudumc has raised discussion about the safety of such controlled malaria infection trials. Based on recommendations of the CCMO and an External Scientific Advisory Committee to the European Malaria Vaccine Development Association, additional safety measures have been added to our protocols, including the early detection of blood infections by PCR followed by swift treatment of trial participants with antimalarial drugs. These safety measures have also been implemented in the current trial. For a more detailed discussion of these events, see section 1.5 and section 12.

10.3.1 Ethical aspects concerning the use of human volunteers

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 [26]. The occurrence of three cardiac events in volunteers participating in phase I/IIa malaria vaccine trials in Nijmegen raised intense discussion about the safety of malaria challenge trials with respect to cardiac events. Based on recommendations of the CCMO and an External Scientific Advisory Committee to the European Malaria Vaccine Development Association, this malaria challenge trial protocol has been adjusted (see also section 1.4).

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 volunteers. Explorative studies looking for new or complementary vaccines are of paramount importance with the potential of large-scale application in endemic countries. These 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 Good Clinical Practices (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 the CCMO the Netherlands and from the funding partner's designated IRB, WIRB.

10.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the research;
2. € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the research;
3. € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.5 Incentives

Enrolled volunteers of phase 1 will receive up to 1000,- Euros in compensation for their time and for the inconveniences of taking part in this study. Volunteers of phase 2 (Group 3) will receive an additional compensation up to 2300,- Euros. Volunteers that will receive a booster immunization and/or a second challenge infection will receive an additional compensation up to 900,- Euros or 1400,- Euros. Infectivity controls will receive up to 800,- Euros. This is based on compensation fees for procedures as below:

- Inconvenience of blood tests and/or visits: 20,- Euros per blood sampling and/or visit
- Mosquito immunization: 400,- Euros
- CHMI: 300,- Euros
- Compensation length: 20,- Euros per month

Travel expenses will not be additionally reimbursed, and compensation will not be provided to volunteers who are not enrolled i.e. screen failures. Eligible volunteers who are enrolled at the inclusion visit as back-ups, but who are not challenged on Day 0 will be compensated 50,- Euros. These compensation amounts are reasonable and in line with Dutch 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 Radboudumc.

11 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

11.1.1 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.

All data will be anonymised; volunteer data will be identified by a unique study number in the CRF. Separate confidential files containing identifiable information will be stored in secured locations. All plasma samples, or other biological samples, with exception of those taken for safety diagnostics, will be labeled with the volunteer study identification number. Samples taken for safety diagnostic (processed by the central clinical laboratory of the Havenziekenhuis) will be labeled with part of the subject identification code, study identification name and a fictitious birth date (only using the subjects actual birth year). The samples will not be labeled with volunteer names or actual birth dates.

The subject identification code will be kept by the principal investigator.

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 volunteers. 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, authorized individuals from PATH, 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.

11.1.2 Data collection

Designated trial staff will enter the data required by the protocol into the electronic CRF (eCRF).

11.1.3 Storage of biological samples

Biological samples, such as immunology samples and leftovers, obtained during the study will be properly stored and governed in a coded manner. Sera and plasma samples will be stored in -80°C freezers and isolated cells in liquid nitrogen. All samples will have a

studycode, subjectcode and a randomizationcode, which enables it to be traced to the human subject. The key to the code is only accessible by the investigators (PI, investigator and co-investigator) and the local safety monitor. Biological samples will be stored for a maximum period of 15 years, after which they will be destroyed. The subjects will be asked for their consent for retention and analysis of the material. Appropriate ethical approval will be obtained prior to future use of stored samples.

11.1.4 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 the ICD-10 terminology.

11.2 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 Malarone® is 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, subject's diaries, 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. The only exception is the data from the quantitative PCR, which is loaded from the PCR machine directly into the eCRF. 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. According to the NFU risk classification system, this clinical trial has been classified as 'middle risk'. 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 25% of included subjects (i.e. 8 subjects).

11.3 Amendments

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

11.4 Annual progress report

The investigator will submit a summary of the progress of the trial to the CCMO once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.
Continuing review reports will be submitted to WIRB, in accordance with its reporting requirements.

11.5 Temporary halt and (prematurely) end of study report

The investigator will notify the CCMO of the end of the study within a period of 8 weeks. The end of the study is defined as the subject's last visit on day 100 after the last exposure to *Pb(PfCS@UIS4)* and 35 days post-CHMI for the infectivity control subjects.
The investigator will notify the CCMO immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the investigator will notify the CCMO within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the CCMO. PATH REC and WIRB will also be notified of any decisions to prematurely suspend or terminate the study, and a study closure report will be submitted to WIRB when all subjects have finished their final visits and follow-up and when the sponsor has indicated the study is closed.

11.6 Public disclosure and publication policy

The study will be publicly disclosed and registered on clinicaltrials.gov before inclusion of the volunteers.The final report will be prepared by the investigators at the Radboudumc, Havenziekenhuis and the ErasmusMC. 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.

12 STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

a. Level of knowledge about mechanism of action

In this study we will investigate the safety and tolerability of administration of a genetically modified P. berghei parasite, Pb(PfCS@UIS4), by mosquito bite.

All pre-clinical data indicate that Pb(PfCS@UIS4) is a safe antigen delivery platform, i.e. not able to develop within human erythrocytes. It is expected that sporozoites of Pb(PfCS@UIS4) are able to invade the liver, but are unable to develop a parasitemia. However, as this is the first in-human trial of Pb(PfCS@UIS4), the exact phenotype of the parasites (sporozoites, liver stages) in humans is unknown. If a break-through blood infection would occur, medical vigilance to ensure early detection of such cases is crucial to the safety of volunteers. Changes in the liver stage development of the genetically modified Pb(PfCS@UIS4) strain may result in prolonged duration of the liver stage or the release of very low numbers of merozoites from the liver, ultimately delaying time to patency. A stringent follow-up schedule until anti-malarial treatment is thus required for all volunteers.

In addition, exposure of humans to a great number of sporozoites/attenuated liver stages parasites could induce an immune response, which may present with clinical symptoms.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

There is extensive clinical experience of controlled human *P. falciparum* malaria infections by the bite of infected mosquitoes. Since 1986 more than 3,500 volunteers have had CHMI by the bites of mosquitoes fed on cultures of *P. falciparum* gametocytes to produce *P. falciparum* sporozoites[26]. This has proved to be a reproducible, predictable and safe method of inducing *P. falciparum* infections. The results of such studies were summarized in 1997 [27], in 2007 [33] and in 2012 [29]. Furthermore, there has been one preceding clinical trial with a genetically modified *Plasmodium falciparum* performed with PfΔp52Δp36 (also refer to Section 1.3) which was administered by a similar route through mosquito bites. In the high dose group (200 bites) all volunteers experienced erythema and pruritus, five experienced edema (of which one >90mm), one experienced induration, one experienced pain. Systemic adverse events were also reported within 7 days after exposure: fever, malaise (both one volunteer, grade 1), headache (grade 2, two volunteers), nausea and vomiting (2 volunteers, grade 2 in one). In the PfΔp52Δp36 trial one volunteer in the high-dose group experienced a breakthrough blood stage infection (confirmed by PCR). The clinical signs and symptoms of this breakthrough blood infection were similar to the wild-type NF54 parental strain.

This is the first-in-human trial of *Pb(PfCS@UIS4)*.

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

Several animal studies and *in vitro* assays have been performed demonstrating the capability of generating an immune response without generating a disease-triggering human blood-stage infection (see D1. Investigator's Brochure).

d. Selectivity of the mechanism to target tissue in animals and/or human beings

The life-cycle of malaria parasites follows a fixed and pre-determined course in the human host. *P. falciparum* sporozoites delivered by mosquito bite are only able to invade into and develop within hepatocytes. These developments are constrained by multiple parasite-host ligand interactions and the parasite's ability to manipulate the host cell's internal environment [36]. It has been shown that the sporozoites of *Pb(PfCS@UIS4)* invade hepatocytes similarly to sporozoites of *P. berghei*, both in *in vitro* cultures of primary hepatocytes and in chimeric mice engrafted with human liver tissue. Furthermore, biodistribution- and toxicology studies have been performed with *Pb(PfCS@UIS4),* and no irregular or unexpected target tissues have been described. Please see D1. Investigator's Brochure for a detailed description.

e. Analysis of potential effect

Safety and tolerability of *Pb(PfCS@UIS4)* will be assessed by recording adverse events, reported at any time during the trial either at a visit or by writing in the diary. Vital signs, including temperature, will be recorded at every visit following exposure. Assessment of complete blood count will be performed at any time point blood is drawn (daily or every other day after exposure), blood chemistry analysis will be performed frequently after exposure. The maximum parasitemia in subjects undergoing CHMIs is equivalent to ~50 parasites/µL, the detection level of thick smear microscopy is ~5 parasites/µL).

In this study we will use thick smear analysis after exposure of Pb(PfCS@UIS4) and qPCR as main tool to detect blood-stage malaria parasites after CHMI, which has an even greater sensitivity (detection limit 20 parasites/ml).

f. Pharmacokinetic considerations

Not applicable.

g. Study population

Included subjects are healthy young adult volunteers, who have been extensively screened for any evidence of co-morbidity, in particular cardiovascular risk factors. Female subjects of child-bearing age are screened for pregnancy by urine test and are required to use contraception throughout the study period. Please refer to 4.2 Inclusion criteria for acceptable forms of contraception. See section 7.7 for procedure to be followed when a volunteer is pregnant during the study.

h. Interaction with other products

Concurrent use of drugs potentially interacting with Malarone® (atovaquone-proguanil) (e.g. artemether-lumefantrine, rifampicin, metoclopramide, oral anti-coagulants and certain anti-retrovirals) are contra-indicated.

i. Predictability of effect

All pre-clinical data indicate that the *Pb(PfCS@UIS4)* sporozoites can invade human hepatocytes but not develop within human erythrocytes. Therefore, it is expected that sporozoites of *Pb(PfCS@UIS4)* are able to elicit an immune respons in the skin and liver needed for pre-erythrocytic protection against *P. falciparum*, without causing disease because of the inability of developing parasitemia.

j. Can effects be managed?

Subjects are frequently seen on an outpatient basis for clinical and parasitological assessment to ensure treatment is started if necessary at the earliest possible time point. In the unlikely event that a break-through blood stage infection occurs, volunteers will be curatively treated with a regimen of Malarone® (atovaquone/proguanil). *Pb(PfCS@UIS4)* has been tested for susceptibility to atovaquone/proguanil *in vitro*, and *in vivo* in mice drugsensitivity studies. Should treatment with this drug need to be discontinued prematurely in any subject for whatever other reason (e.g. intolerability), artemether/lumefantrine will act as a back-up drug, and is a good alternative. Artemether/lumefantrine has also been shown to be effective against *Pb(PfCS@UIS4)* in drug sensitivity studies (see D1 Investigator's Brochure).

12.2 Synthesis

The extensive pre-clinical evaluation, indicate that Pb(PfCS@UIS4) can be safely used in healthy volunteers in this trial and the risks are therefore considered low. They will, furthermore, be minimized by strict adherence to the inclusion/exclusion criteria and close clinical monitoring.

13 Appendices

13.1 Appendix 1: Systematic Coronary Risk Evaluation (SCORE) Risk Chart

The SCORE risk chart, made by the European Society of Cardiology, comprised of gender, age, total cholesterol, systolic blood pressure and smoking status, with relative risk chart, qualifiers and instructions.

For the Netherlands, the low risk chart (Figure 1) is used. To use this chart, we should find the cell nearest to a person's age, cholesterol, and systolic blood pressure values. The risk in the chart is 10-year total risk.

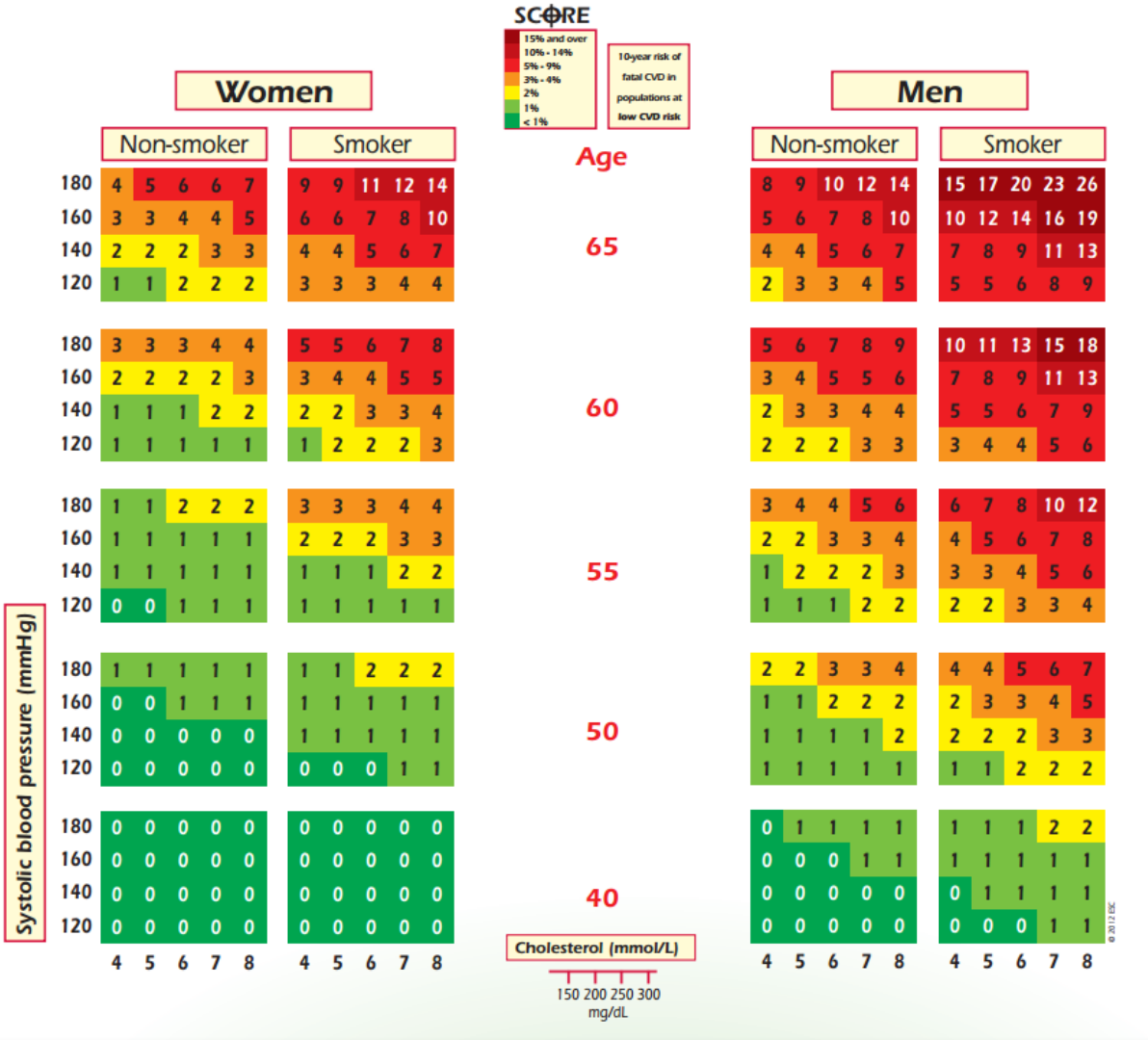

Figure 1. SCORE – European Low Risk Chart

13.2 Appendix 2: Classification of Adverse Events

The following tables are adapted from the Food and Drug Administration's Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Trials.

A. Tables for Clinical Abnormalities

Local Reaction to mosquito bites	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Pain	Does not interfere with activity	Repeated use of nonnarcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest
Pruritis	No interference with activity	Some interference with activity	Significant; prevents daily activity
Induration/Swelling *	<15 cm and does not interfere with activity	≥15 cm or interferes with activity	> 15 cm or prevents daily activity

*Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement

Vital signs	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Fever (^{o}C)	38.0 – 38.4	38.5 – 38.9	≥39.0
Tachycardia – beats per minute	101 – 115	116 – 130	>130
Bradycardia – beats per minute	50 – 54	45 – 49	< 45
Hypertension (systolic) –mmHg	141 – 150	151 – 155	>155
Hypertension (diastolic) – mmHg	91 – 95	96 – 100	>100
Hypotension (systolic) – mmHg	85 – 89	80 – 84	< 80
Respiratory rate – breaths per minute	17 – 20	21 – 25	>25

* Subject should be at rest for all vital sign measurements.

** Auricular temperature.

*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Rash	No interference with activity	Some interference with activity	Significant; prevents daily activity
Urticaria	No interference with activity	Some interference with activity	Significant; prevents daily activity
Pruritis	No interference with activity	Some interference with activity	Significant; prevents daily activity
Edema	No interference with activity	Some interference with activity	Significant; prevents daily activity
Headache	No interference with activity	Repeated use of nonnarcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity
Malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity
Rigors	No interference with activity	Some interference with activity	Significant; prevents daily activity

Myalgia	No interference with activity	Repeated use of nonnarcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity
Arthralgia	No interference with activity	Repeated use of nonnarcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity
Nausea/Vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration
Abdominal pain	No interference with activity	Repeated use of nonnarcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration
Chest pain	No interference with activity	Repeated use of nonnarcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity
Palpitations	No interference with activity	Some interference with activity	Significant; prevents daily activity
Shortness of breath	No interference with activity	Some interference with activity	Significant; prevents daily activity

B. Tables for Laboratory Abnormalities

Haematology		**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Erythrocytes ($.10^{12}$/L)	Male	4.0 - 4.4	3.5 – 3.9	< 3.5
	Female	3.5 – 3.9	3.0 – 3.4	< 3.0
Hematocrit (%)	Male	0.35 – 0.39	0.30 – 0.34	< 0.30
	Female	0.30 – 0.34	0.25 – 0.29	< 0.25
Hemoglobin (mmol/L)	Male	8.0 – 8.5	7.0 – 7.9	< 7.0
	Female	7.0 – 7.5	6.0 – 6.9	< 6.0
MCV (fL)		60 – 79	40 – 59	< 40

MCH (fmol)		1.50 – 1.69	1.25 – 1.49	< 1.25
MCHC (mmol/L)		15.0 – 18.9	10.0 – 14.9	< 10
Leukocytes ($.10^9$/l)	Leukocytosis	11.01 – 15.00	15.01 – 20.00	> 20.00
	Leukopenia	3.00 – 3.99	2.00 – 2.99	< 2.00
Thrombocytopenia ($.10^9$/l)		75 – 150	50 – 74	< 50

Serum		**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Alkaline phosphatase		1.1 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN
ALT		1.1 – 2.5 x ULN	2.6 – 15.0 x ULN	> 15.0 x ULN
AST		1.1 – 2.5 x ULN	2.6 – 15.0 x ULN	> 15.0 x ULN
Bilirubin (Total)		1.1 – 1.25 x ULN	1.26 – 3 x ULN	> 3 x ULN
Creatinine (umol/L)	Male	105 – 123	124 – 142	> 142
	Female	91 – 109	110 – 128	> 128
Gamma GT		1.1 – 2.5 x ULN	2.6 – 15.0 x ULN	> 15 x ULN
Potassium – Hyperkalemia (mmol/L)		5.1 – 5.2	5.3 – 5.4	> 5.4
Potassium – Hypokalemia (mmol/L)		3.3 - 3.4	3.1 – 3.2	< 3.1
Sodium - Hypernatremia (mmol/L)		146 – 147	148 – 149	> 149
Sodium - Hyponatremia (mmol/L)		132 – 134	131 – 130	< 130
Ureum		22 – 25	26 – 29	> 29

*ULN is the upper limit of the normal range.

14 REFERENCES

1. Luke, T.C. and S.L. Hoffman, *Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated Plasmodium falciparum sporozoite vaccine.* J Exp Biol, 2003. **206**(Pt 21): p. 3803-8.
2. Roestenberg, M., et al., *Protection against a malaria challenge by sporozoite inoculation.* N Engl J Med, 2009. **361**(5): p. 468-77.
3. Organization, W.H., *WHO Malaria Report 2015*. 2015.
4. Murray, C.J., et al., *Global malaria mortality between 1980 and 2010: a systematic analysis.* Lancet, 2012. **379**(9814): p. 413-31.
5. Doolan, D.L., C. Dobano, and J.K. Baird, *Acquired immunity to malaria.* Clin Microbiol Rev, 2009. **22**(1): p. 13-36, Table of Contents.
6. Beeson, J.G., F.H. Osier, and C.R. Engwerda, *Recent insights into humoral and cellular immune responses against malaria.* Trends Parasitol, 2008. **24**(12): p. 578-84.
7. Pombo, D.J., et al., *Immunity to malaria after administration of ultra-low doses of red cells infected with Plasmodium falciparum.* Lancet, 2002. **360**(9333): p. 610-7.
8. Hoffman, S.L., et al., *Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites.* J Infect Dis, 2002. **185**(8): p. 1155-64.
9. Crompton, P.D., S.K. Pierce, and L.H. Miller, *Advances and challenges in malaria vaccine development.* J Clin Invest, 2010. **120**(12): p. 4168-78.
10. Organisation, W.H. *Tables of malaria vaccine projects globally*. 2016; Available from: http://www.who.int/vaccine_research/links/Rainbow/en/index.html.
11. Abdulla, S., et al., *Safety and immunogenicity of RTS,S/AS02D malaria vaccine in infants.* N Engl J Med, 2008. **359**(24): p. 2533-44.
12. Breman, J.G. and C.V. Plowe, *A malaria vaccine for control: more progress.* J Infect Dis, 2009. **200**(3): p. 317-20.
13. Rts, S.C.T.P., et al., *A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants.* N Engl J Med, 2012. **367**(24): p. 2284-95.
14. Targett, G.A. and B.M. Greenwood, *Malaria vaccines and their potential role in the elimination of malaria.* Malar J, 2008. **7 Suppl 1**: p. S10.
15. Nussenzweig, R.S., et al., *Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei.* Nature, 1967. **216**(5111): p. 160-2.
16. Nganou-Makamdop, K. and R.W. Sauerwein, *Liver or blood-stage arrest during malaria sporozoite immunization: the later the better?* Trends Parasitol, 2013. **29**(6): p. 304-10.
17. Khan, S.M., et al., *Genetic engineering of attenuated malaria parasites for vaccination.* Curr Opin Biotechnol, 2012. **23**(6): p. 908-16.
18. Spring, M., et al., *First-in-human evaluation of genetically attenuated Plasmodium falciparum sporozoites administered by bite of Anopheles mosquitoes to adult volunteers.* Vaccine, 2013. **31**(43): p. 4975-83.
19. Roestenberg, M., et al., *Long-term protection against malaria after experimental sporozoite inoculation: an open-label follow-up study.* Lancet, 2011. **377**(9779): p. 1770-6.
20. Sauerwein, R.W., M. Roestenberg, and V.S. Moorthy, *Experimental human challenge infections can accelerate clinical malaria vaccine development.* Nat Rev Immunol, 2011. **11**(1): p. 57-64.
21. Laurens, M.B., et al., *A consultation on the optimization of controlled human malaria infection by mosquito bite for evaluation of candidate malaria vaccines.* Vaccine, 2012. **30**(36): p. 5302-4.
22. Kumar, K.A., et al., *Conserved protective mechanisms in radiation and genetically attenuated uis3(-) and uis4(-) Plasmodium sporozoites.* PLoS One, 2009. **4**(2): p. e4480.
23. Nussenzweig, V. and R.S. Nussenzweig, *Development of a sporozoite malaria vaccine.* Am J Trop Med Hyg, 1986. **35**(4): p. 678-88.

24. Mauduit, M., et al., *Minimal role for the circumsporozoite protein in the induction of sterile immunity by vaccination with live rodent malaria sporozoites.* Infect Immun, 2010. **78**(5): p. 2182-8.
25. Gruner, A.C., et al., *Sterile protection against malaria is independent of immune responses to the circumsporozoite protein.* PLoS One, 2007. **2**(12): p. e1371.
26. Chulay, J.D., et al., *Malaria transmitted to humans by mosquitoes infected from cultured Plasmodium falciparum.* Am J Trop Med Hyg, 1986. **35**(1): p. 66-8.
27. Church, L.W., et al., *Clinical manifestations of Plasmodium falciparum malaria experimentally induced by mosquito challenge.* J Infect Dis, 1997. **175**(4): p. 915-20.
28. Epstein, J.E., et al., *Malaria vaccines: are we getting closer?* Curr Opin Mol Ther, 2007. **9**(1): p. 12-24.
29. Roestenberg, M., et al., *Comparison of clinical and parasitological data from controlled human malaria infection trials.* PLoS One, 2012. **7**(6): p. e38434.
30. Hermsen, C.C., et al., *Testing vaccines in human experimental malaria: statistical analysis of parasitemia measured by a quantitative real-time polymerase chain reaction.* Am J Trop Med Hyg, 2004. **71**(2): p. 196-201.
31. Nieman, A.E., et al., *Cardiac complication after experimental human malaria infection: a case report.* Malar J, 2009. **8**: p. 277.
32. van Meer, M.P., et al., *Idiopathic acute myocarditis during treatment for controlled human malaria infection: a case report.* Malar J, 2014. **13**: p. 38.
33. Epstein, J.E., et al., *Safety and clinical outcome of experimental challenge of human volunteers with Plasmodium falciparum-infected mosquitoes: an update.* J Infect Dis, 2007. **196**(1): p. 145-54.
34. Verhage, D.F., et al., *Clinical outcome of experimental human malaria induced by Plasmodium falciparum-infected mosquitoes.* Neth J Med, 2005. **63**(2): p. 52-8.
35. van Wolfswinkel, M.E., et al., *Changes in total and differential leukocyte counts during the clinically silent liver phase in a controlled human malaria infection in malaria-naive Dutch volunteers.* Malar J, 2017. **16**(1): p. 457.
36. Gaur, D. and C.E. Chitnis, *Molecular interactions and signaling mechanisms during erythrocyte invasion by malaria parasites.* Curr Opin Microbiol, 2011. **14**(4): p. 422-8.