Clinical Trial Protocol

A randomized, controlled, double-blind, single-center phase 1 clinical trial to evaluate safety, tolerability, immunogenicity and efficacy of CAF01 and aluminum hydroxide as adjuvants for the malaria vaccine candidate GMZ2 in healthy adult African volunteers

GMZ2CAF01

Version 1.1
April 24, 2015

Clinical Trial Sponsor: Centre de Recherches Médicales de Lambaréné
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1. Administrative issues

Title	A randomized, controlled, double-blind, single-center phase 1 clinical trial to evaluate safety, tolerability, immunogenicity and efficacy of CAF01 and aluminum hydroxide as adjuvants for the malaria vaccine candidate GMZ2 in healthy adult African volunteers
Study code	GMZ2CAF01
Development phase	1
Protocol version, date	Version 1.1, 24 April 2015
Amendments	None
Investigational products	GMZ2 formulated in CAF01 GMZ2 formulated in aluminum hydroxide
Control vaccine	Rabies vaccine
Challenge product	Aseptic, purified, vialed, cryopreserved, infectious *Plasmodium falciparum* sporozoites, strain NF54, produced by Sanaria Inc. (PfSPZ Challenge).
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Regulatory review	Direction Générale de la Santé, Ministère de la Santé, Gabon
Funding	German Centre for Infection Research Bundesministerium für Bildung und Forschung (BMBF), Germany

2. Modification history

Version	Date	Author
Draft	20MAY2014	B. Mordmüller, G. Surat, U. Ateba Ngoa
Version 1	22DEC2014	B. Mordmüller
Version 1.1	24APRIL2015	U. Ateba Ngoa, B. Mordmüller

2.1 Amendment details

Amendment number	1
Protocol version	1.1
Protocol date	24. April 2015
Amendment date	24. April 2015
Author	U. Ateba Ngoa
Summary and location of first appearance of changes	**1.** Condensing of the vaccination schedule: *Page 11* **2.** Increase in frequency of urine collection: *Page 36* **3.** Expansion of exploratory endpoint to include testing of existing blood samples: *Page 11* 4. Temperature to be measured with an axillary thermometer instead of tympanic: *Page 30* 5. Timeframe after vaccination when CHMI will be performed extended: *Page 12* 6. Correction to study group E: *Page 28* 7. Addition of III+28 visit to assess immunogenicity at D84: *Page 38* 8. Shortening of follow up period after CHMI: *Page 13 and 40*
Rationale for changes	1. In a practical manner it was not possible to respect the interval of 7 days between the slot 2 and slot 3 and between slot 3 and slot 4 when we administered the first dose of the vaccine. Therefore we would like the vaccine injection to be done as follow for Dose 2 and Dose 3: Study day 0: A (n = 2), B (n = 3), C (n = 1) Study day 1: A (n = 2), B (n = 3), C (n = 5), D (n = 1) Study day 2: A (n = 2), B (n = 3), C (n = 1), D (n = 5), E (n = 5) Study day 3: A (n = 2), B (n = 3), C (n = 1), D (n = 6), E (n = 5) This change happened because we observed a delay in obtaining the authorisation from the Ministry of Health to start the study. The study vaccine is due to expire on the 19th of June 2015, so we had to complete the administration of the first dose of vaccine by the 24th April 2015. More efforts were engaged to ensure that the safety of the participants was preserved. After vaccine administration participants stayed on site for a longer period. In addition they were phoned by a study physician to make sure that they did not experience any serious AEs at home. Finally they visited the clinic the day after vaccination where they were examined by a physician.

	2. In order to better assess the effect of the vaccination and the PfSPZ challenge on the metabolism of the study participants we would like to increase the timepoint for urine collection. Urine will therefore be collected at days: Day I1, Day III and III1, Day C-7 to C-1, Day C1 to C5, D34 or at day of malaria symptoms and C56. 3. In order to assess the effect of filaria parasite on the study vaccine we would like to determine whether GMZ2CAF01 study participants are infected with either Loa loa or Mansonella perstans. This assessment will be done using the Leucoconcentration method on 1 ml of blood collected in an EDTA tube. We do not plan to increase the number of blood to be collected. Rather we will use the left over blood taken at one of the visit comprises between visit I and III. Therefore the following exploratory change: "Assess the effect of microbial composition of stool and urine on vaccine-induced immune responses" To "Assess the effect of microbial composition of blood, stool and urine on vaccine-induced immune responses" 4. A tympanic thermometer was unable to be sourced, therefore an axillary thermometer was used. 5. In order to ensure sufficient trained staff would be available, the timeframe in which CHMI after vaccination will be performed has been extended. This does not alter the outcomes of the trial, but means the investigators have the flexibility to ensure participant safety by planning the CHMI for an optimal time with regards to available resources. 6. Study Group E was incorrectly written in the protocol as receiving GMZ2-Alum. It has been corrected to GMZ2-CAF01. 7. In previous GMZ2 studies, immunogenicity was assessed at D84. In this amendment, D84 no longer corresponds with visit C-7 which is when immunogenicity was planned to be assessed. For this reason, an additional III+28 visit has been added to allow immunogenicity testing at the appropriate time, providing comparable results with previous trials. The planned procedures of visit C-7 have been moved to visit III+28 and will not be repeated at C-7. 8. The planned 140 days of follow up after CHMI would require a long commitment from patients and this has been shortened to 56 days. This time frame is considered sufficient to ensure health and safety of the participants, and the additional 3 months would

	confer no further benefit to the trial data or the participants.

3. Signatures

3.1. Sponsor signatory approval

Signature	Date
(Bertrand Lell)	

3.2. Investigator agreement

The signatures below constitute the approval of this protocol and the attachments, and provide the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and ICH guidelines.

Signature	Date
(Ulysse Ateba Ngoa)	
(Ayola Akim Adegnika)	
(Benjamin Mordmüller)	

3.3. Confidentiality statement

This document contains confidential information that must not be disclosed to anyone other than the trial sponsor, the Investigator team, and members of the ethical and regulatory review boards. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the principal Investigator (Ulysse Ateba Ngoa).

4. Abbreviations

ADCI	Antibody Dependent Cellular Inhibition
AE	Adverse Event
AEFI	Adverse Event Following Immunization
ALS	Advanced Life Support
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BSC	Biological Safety Cabinet
CAF01	Cationic adjuvant formulation 01
CERMEL	Centre de Recherches Médicales de Lambaréné
CBC	Complete Blood Count
CRF	Case Report Form
DDA	N,N-dimethyl-N,N-dioctadecylammonium
DVI	Direct venous inoculation
EC	Ethics Committee
ELISA	Enzyme-linked Immunosorbent Assay
GCP	Good Clinical Practice
GI	Growth inhibition
GLURP	*Plasmodium falciparum* glutamate rich protein
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B Surface Antigen
HCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IM	Intramuscular
IV	Intravenous
LDH	Lactate Dehydrogenase
LSM	Local Safety Monitor
MSP3	*Plasmodium falciparum* merozoite surface protein 3
NaCl	Sodium chloride
PCR	Polymerase Chain Reaction
P. falciparum, Pf	*Plasmodium falciparum*
PfSPZ	*P. falciparum* sporozoites
PI	Principal Investigator
Q1, Q2, Q3, Q4	1st, 2nd, 3rd, 4th quarter of the year
qPCR	Quantitative PCR
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SPZ	Sporozoite
SSI	Statens Serum Institut
SUSAR	Suspected Unexpected Serious Adverse Reaction
UKT	Universitätsklinikum Tübingen
WHO	World Health Organization

5. Synopsis

Title	A randomized, controlled, double-blind, single-center phase 1 clinical trial to evaluate safety, tolerability, immunogenicity and efficacy of CAF01 and aluminum hydroxide as adjuvants for the malaria vaccine candidate GMZ2 in healthy adult African volunteers
Study code	GMZ2CAF01
Study population	Healthy adult males with a history of exposure to malaria, aged between 18 and 40 years
Investigational product	Recombinant GMZ2, expressed in *Lactococcus lactis* adjuvanted in cationic adjuvant formulation 01 (CAF01) Recombinant GMZ2, expressed in *Lactococcus lactis* adjuvanted in aluminum hydroxide (alum)
Control vaccine	Rabies vaccine
Challenge product	Aseptic, purified, vialed, cryopreserved, infectious *Plasmodium falciparum* sporozoites, strain NF54, produced by Sanaria Inc. (PfSPZ Challenge).
Route	Vaccination: Intramuscular (IM); Challenge: Direct venous inoculation (DVI)
Rationale	A malaria vaccine would ideally complement current malaria control efforts. Three strategies to interfere with parasite development are pursued: 1) elicit immune responses against pre-erythrocytic stages of parasite development (sporozoite and hepatic stages), 2) mimic naturally acquired immunity against asexual blood stage and 3) inhibiting transmission through immune responses against gametocytes. Most advanced malaria vaccine candidates belong to the pre-erythrocytic and asexual blood stage type of vaccines. During asexual replication in the blood, symptoms and complications may occur, whereas the other stages are clinically silent. Naturally acquired immunity is primarily directed against the asexual blood stage and very robust, although usually not sterilizing. An effective vaccine against asexual blood stage parasites would prevent malaria and, if only partially efficacious (as naturally acquired immunity), has the potential to improve the clinical outcome of the infection. Naturally acquired immunity is acquired after repeated exposure to the parasite and develops slowly. Reducing the number of infections during childhood using a highly effective, pre-erythrocytic stage-targeting intervention may lead to a rebound of morbidity and mortality later in life. Hence, apart from the direct benefits of a blood stage vaccine, it would be an almost ideal combination partner for interventions that are prone to rebound morbidity (e.g. pre-erythrocytic malaria vaccines). GMZ2 is an asexual blood stage candidate that was developed by the Statens Serum Institut, Denmark (SSI) and its partners. It is a fusion protein of conserved fragments of *Plasmodium falciparum* merozoite surface protein 3 (MSP3) and glutamate rich protein (GLURP). The antigen was selected based on sero-epidemiological and functional studies and showed excellent safety, tolerability and good immunogenicity when adjuvanted with alum. Results of three phase 1 studies on GMZ2-alum led to the decision to perform a phase 2 clinical trial in African children to assess safety, tolerability and efficacy in the target population. Results of the trial are still pending but preliminary data support the very good safety and tolerability profile of GMZ2 while efficacy seems modest. In general, data from the four trials suggest that an improved and more durable immune response

	against the vaccine antigen will result in significantly better protection. Therefore combination of GMZ2 with a novel and more potent adjuvant is the rational next step in clinical development. CAF01 is a novel adjuvant, which was developed by SSI and several clinical trials in Europe and Africa demonstrated good safety, tolerability and improved immunogenicity when combined with various vaccine candidates. GMZ2CAF01 is performed to compare safety, tolerability, immunogenicity and efficacy of GMZ2 formulated with CAF01 to a control vaccine (Rabies) and 100 μg GMZ2 in alum, the best studied GMZ2 regimen so far, with approximately 1000 vaccinated individuals. Efficacy will be tested by controlled human malaria infection (CHMI) by direct venous inoculation (DVI) of 3200 PfSPZ Challenge, five to eighteen weeks after completion of the vaccination regimen.
Objectives	**Primary** Establish a regimen of GMZ2-CAF01, which is safe and well tolerated **Secondary** Establish a regimen of GMZ2-CAF01 that reduces parasite multiplication upon controlled human malaria infection (CHMI) Establish a regimen of GMZ2-CAF01 that induces a superior anti-GMZ2 antibody-response compared to GMZ2-alum and a control vaccine (Rabies) **Exploratory** Assess functional activity of vaccine-induced antibodies Assess the effect of microbial composition of blood, stool and urine on vaccine-induced immune responses Assess the effect of metabolites in blood and urine, measured by non-biased methods, on vaccine-induced immune responses
Immunizations	All immunizations will be given as three IM injections in the deltoid muscle on alternating sides in four-week intervals. Regimens are: **A**: Three times Rabies vaccine IM **B**: Three times 100 μg GMZ2 in alum IM **C**: Three times 30 μg GMZ2 in CAF01 IM **D**: Three times 100 μg GMZ2 in CAF01 IM **E:** Three times 100 μg GMZ2 in CAF01 IM without subsequent CHMI
Challenge	Participants of Groups A, B, C and D will receive CHMI with 3200 PfSPZ Challenge five to eighteen weeks after completion of the immunization regimen. PfSPZ Challenge is injected intravenously and participants are monitored daily up to 35 days following injection. One week before PfSPZ administration participants are treated with clindamycin. All participants are treated with a registered antimalarial 35 days after PfSPZ Challenge injection or when they develop malaria, whatever comes first.
Study design	GMZ2CAF01 is a double-blinded, randomized, controlled, single-center phase 1 clinical trial. Participants will receive three times control vaccine (n = 8; Group A), 100 μg GMZ2 formulated in alum (n = 12; Group B), 30 μg GMZ2 formulated in CAF01 (n = 8; Group C) or 100 μg GMZ2 formulated in CAF01 (n = 22; Groups D and E). GMZ2 dose escalation in the CAF01 groups will be staggered over four days with interim safety assessments and small sentinel groups of the next higher GMZ2-CAF01 dose.

	CHMI: Participants of groups A, B, C and D will receive PfSPZ Challenge five to eighteen weeks after completion of the vaccination regimen.
Definitions	**AE**: Adverse Event. Any untoward medical occurrence in a participant after inclusion in the trial. **AE of special interest**: Malaria during immunization and follow up. **AEFI**: AE following immunization. In this study all AEs that occur between first injection and four weeks after the last injection. **Malaria**: *P. falciparum* asexual blood stage parasitemia detected by microscopy and at least one symptom that can be associated to malaria. **Safety**: Occurrence and relationship to vaccination of serious adverse events (SAE) and occurrence, intensity and relationship of any solicited and unsolicited adverse event (AE) during the entire study period in all participants.
Safety	The study may be placed on safety hold for the following reasons: • On advice of the safety monitor • On advice of the Investigators • On advice of the ethics committee or the safety monitoring committee (SMC) • One or more participants experience a SAE that is at least possibly related to GMZ2 or PfSPZ Challenge administration • Two or more grade 3 adverse events in the same group of participants, which are at least possibly related to GMZ2 or PfSPZ Challenge administration The study will be stopped for the following reason: • One or more participants experience a Serious Adverse Reaction to GMZ2 or PfSPZ Challenge.
Endpoints	**Primary safety endpoint** Number or occurrence of at least possibly related Grade 2 AEs, Grade 3 AEs and SAEs from time of first vaccine injection to the end of the follow-up period. **Secondary safety endpoint** Number of at least possibly related local, systemic and unsolicited AEs from time of first vaccine injection. **Primary efficacy endpoint** Time to malaria from DVI of PfSPZ Challenge **Secondary efficacy endpoint** Time to asexual blood stage parasitemia from DVI of PfSPZ Challenge **Primary immunogenicity endpoint** Difference in the baseline-corrected area under the curve anti-GMZ2 antibody concentration.
Sample size	Total sample size is 50 participants. Group A (Rabies vaccine): n = 8 Group B (100 μg GMZ2 in alum): n = 12

	Group C (30 μg GMZ2 in CAF01): n = 8 Group D (100 μg GMZ2 in CAF01): n = 12 Group E (100 μg GMZ2 in CAF01): n = 10
Follow up	Two months following CHMI.
Trial period	Total trial period: April 2015 – January 2016 Approximate individual participant's time in the trial: 280 days

6. Background and rationale

6.1. Epidemiology of malaria

Malaria is an infectious disease caused by parasites of the genus *Plasmodium*. It remains one of the world's greatest public health challenges with about 207 million cases in 2012 and an estimated 627,000 deaths worldwide [1]. Most of these deaths occur among sub-Saharan African children under the age of five, but almost 50% of the world population remains at risk of malaria [1]. In 2013, 97 countries and areas had ongoing malaria transmission, including large areas of Africa, Central and South America, parts of the Caribbean, Southeast Asia and the Middle East [1].

There are five parasite species causing malaria in humans: *P. falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale* and *P. vivax*. *P. knowlesi* has only been described recently to infect humans as it was only known as being prevalent amongst monkeys in Southeast Asia [2, 3].

P. falciparum remains the most important and deadly malaria parasite amongst the five species [1]. Repeated *P. falciparum* malaria episodes lead to immunity against severe forms and complications of the disease [1, 4–7]. This partial immunity was dubbed semi-immunity and leaves children of highly endemic areas as the most vulnerable target [1, 4, 8].

Infected *Anopheles* mosquitoes serve as malaria vectors. About 460 species of *Anopheles* are recognized, varying in life-span, biting habit and breeding preferences, however only a few dozen of these species are relevant to the transmission of malaria [9]. Most of the *Anopheles* species with high vector competence live in the tropics and find an ideal habitat in many parts of Africa.

6.2. Lifecycle of the malaria parasite

The lifecycle of the parasites that cause malaria in humans is complex, with stages in both human and mosquito hosts (Figure 1). The bites of infected female *Anopheles* mosquitoes transmit malaria sporozoites (SPZ) to the human host [1][1] where they travel via the bloodstream to the liver and invade hepatocytes [2, liver-stage]. Here, they mature into merozoites for 5.5 to 7.5 days [3] after which the infected hepatocyte releases a large number of merozoites into the bloodstream [4]. Merozoites then invade erythrocytes where they multiply and after 2 days egress from the erythrocyte, releasing progeny merozoites that in turn invade new erythrocytes [5, blood-stage]. This results in a feed-forward loop with exponential growth of the parasite population until the immune system or metabolic resource-restriction dampens amplification. A small percentage of merozoites differentiate into gametocytes [6], which, when ingested by a mosquito, mature to gametes. Subsequently, gametes of the opposite sex unite to create zygotes [7, 8]. The zygote matures to an ookinete and then an oocyst, which releases sporozoites that migrate to the mosquito's salivary glands [9] and are injected into the human host when the mosquito feeds [10]. Sporozoites, gametocytes, and the liver-stage of malaria parasites do not induce pathology, symptoms or signs of malaria. It is the asexual blood-stage of infection that is associated with symptoms and potentially severe or fatal complications, and it is this stage of the parasite lifecycle that is targeted by vaccination with GMZ2.

[1] Numbers in square brackets correspond to labels in Figure 1.

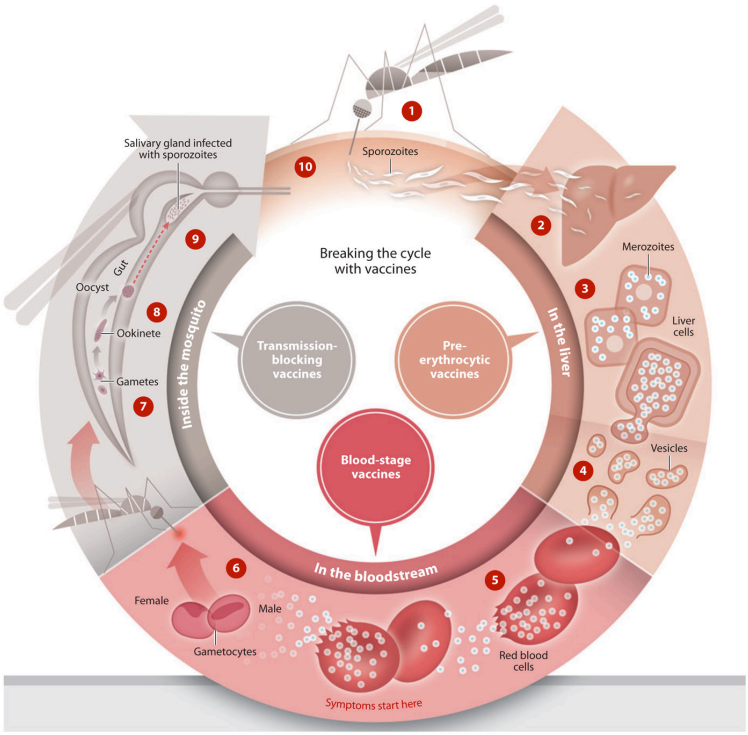

Figure 1: Lifecycle of malaria highlighting the three major stages that are targets for vaccine-development (from: malaria vaccine initiative; www.malariavaccine.org)

6.3. Malaria in Lambaréné, Gabon

Lambaréné is the Capital of Moyen Ogooue, which is one of 9 provinces of Gabon. It is located near the equator in the central African rain forest. Climate conditions are mostly steady, with slightly less rainfall from June to August. The major vectors for malaria transmission in Gabon are *Anopheles gambiae* and *A. moucheti*. The entomological inoculation rate (EIR) in Lambaréné and its surroundings is about 50 infective bites per person per year [10]. There is little seasonal variability in transmission rates or parasite prevalence. *P. falciparum* is the predominant species and responsible for 95% of all infections [11]. *P. falciparum* parasitemia with clinical symptoms is infrequent in adults, and occurs almost solely in school and pre-school aged children. The level of chloroquine resistance is close to 100% both *in vitro* and *in vivo*. Data from the Severe Malaria in African Children (SMAC) studies show that there is a fairly constant annual number of children admitted to the ward with *P. falciparum*, averaging about 500 children per year [10]. On average, children aged 2-12 years experience about 1.5 malarial attacks per year, with a large variability among individuals [12]. Anemia is the most common complication with 16% of all hospitalized malaria cases presenting severe, life-threatening anemia [13].

6.4. Malaria vaccines

A malaria vaccine would be the first anti-parasitic vaccine for human use. The history of attempts to develop such a vaccine dates back to 1910, when the first vaccination experiments using attenuated sporozoites were reported [14]. Highly efficacious vaccines may contribute enormously to disease control but for current malaria vaccine candidates an integrated approach is more likely to result in substantial health benefits [15, 16]. Most malaria vaccine candidates in advanced clinical development to date have failed to show high efficacy or efficacy at all. A notable exception is RTS,S; a pre-erythrocytic vaccine candidate which entered clinical phase 3

and showed 56% efficacy in a first analysis [17], and more recently 31% clinical efficacy when integrated into an expanded program on immunization schedules for infants [18].
Other vaccine candidates in clinical trials in their respective target populations include those designed to mimic naturally acquired immunity against asexual blood-stage parasites. These vaccine antigens include sequences from merozoite surface proteins (MSP) 1, 2 and 3 [19–21], and apical membrane antigen (AMA) 1 (23). GMZ2 is a fusion protein of fragments of *P. falciparum* glutamate rich protein (GLURP) and merozoite surface protein 3 (MSP3) and belongs to the class of vaccines intended to induce immunity against the asexual blood-stage to control parasitemia, thereby preventing clinical complications and death.
Naturally acquired immunity is only developed following repeated infections with *P. falciparum* [4, 6, 7]. It does not always prevent disease but provides robust protection against massive parasite replication, severe malaria and complications. In general, adults from endemic regions will only develop mild, uncomplicated forms of malaria and sometimes maintain low-grade parasitemia without symptoms over long periods of time [6]. Since naturally acquired immunity does not protect against infection and requires repeated exposure to blood-stage parasites, it was termed semi-immunity – a somewhat ambiguous term since it suggests that immunity is not robust, which is not the case [22, 23]. Passive transfer experiments in humans revealed that antibodies are central mediators of semi-immunity and several independent studies have shown that transfer of antibodies, purified from clinically immune individuals, can be used therapeutically, even in patients with severe malaria [24, 25]. The experiments were reproduced and expanded later, when it was shown that purified IgG from semi-immune Africans were able to control parasitemia of patients who acquired infection in Southeast Asia [26]. *In vitro* investigations with the same protective IgG preparations demonstrated that antibodies, on their own, do not substantially inhibit parasite growth, but act synergistically with blood mononuclear cells to control parasite multiplication. This parasite containing mechanism is referred to as Antibody-Dependent Cellular Inhibition (ADCI) [27].

6.5. GMZ2

GMZ2 is a recombinant fusion protein between fragments of *P. falciparum* GLURP and MSP3 expressed in *Lactococcus lactis*. Fragments contained in GMZ2 are the c-terminal end of MSP3 (amino acids 212-380) and the R0 region of GLURP (amino acids 27–500). Both peptides are conserved between *P. falciparum* strains and are available as single peptides to assess interaction of antibody responses.
The *L. lactis* system has been established for production of recombinant proteins under current Good Manufacturing Practice (cGMP). As a production host, *L. lactis* has several advantages over more commonly used prokaryotic hosts such as *Escherichia coli*, because it allows efficient secretion of the recombinant protein into the culture supernatant and because *L. lactis* is a Gram-positive organism, which does not produce endotoxins. The GLURP-MSP3 fusion protein provides an adequate presentation of GLURP and MSP3 epitopes. A summary of non-clinical and stability studies is provided in the IB.
GMZ2 has good immunogenicity when administered with an adjuvant and immune responses against the fusion protein are higher compared to administration of the single peptides [28].

6.5.1. Immuno-epidemiological studies

Under natural exposure, individuals can develop antibodies against GLUPR, MSP3 and the vaccine antigen, even in low transmission settings [29] and concentration of GLURP- and MSP3-specific antibodies are associated with protection against clinical malaria in Southeast Asia [30] and sub-Saharan Africa [31]. A systematic review and meta-analysis of published immune-epidemiological data on antibody-reactivity against merozoite proteins supports the development of MSP3 and GLURP as vaccine candidate [32], although such association studies are prone to bias since causality is not assessed.

Several studies have shown that cytophilic (primarily IgG3) anti-MSP3 and -GLURP antibodies are mostly associated with protection [30, 33, 34] and that direct growth inhibition plays a minor functional role. However, purified antibodies and sera from semi-immune individuals inhibit parasite growth in the presence of immune cells (monocytes). This antibody-dependent cellular inhibition (ADCI) has been used to identify novel vaccine antigens (e.g. MSP3 [35]), validate candidates (e.g. GLURP [36]) and assess functionality of vaccine-induced antibodies [37].

6.5.2. Non-clinical studies

Preclinical development of GMZ2 included a set of studies to assess antigenicity (the ability to be recognized by naturally acquired antibodies), immunogenicity of GMZ2 and the single MSP3 and GLURP peptides as well as safety and protective efficacy. A formal pre-clinical assessment of GMZ and the adjuvant systems was performed by certified contract research organizations (CRO) and is described in the Investigator's Brochure.

Antigenicity

The antigenicity of the GMZ2 and the constituting MSP3 and GLURP fragments was evaluated by enzyme linked immunosorbent assay (ELISA) with sera from semi-immune individuals. One set of experiments used serially diluted plasma from 71 Liberian adults [28]. Concentration of specific antibodies against MSP3 and GLURP varies substantially between individuals and anti-GMZ2-reactivity is generally higher than against the single peptides. Results were reproduced in subsequent studies, including a phase 1 clinical trial in Gabon [38].

Immunogenicity

To assess immunogenicity of GMZ2 and its constituents (MSP3 and GLURP) mice were immunized by subcutaneous injection of antigen preparations (MSP3, GLURP, mixture of MSP3 plus GLURP, or GMZ2) adjuvanted with Montanide ISA720. Immunogenicity of GLURP was not strongly affected by the antigen preparation, whereas MSP3-antibody concentration was ~4-fold higher when administered as fusion protein (GMZ2) [28]. Depending on adjuvant and formulation, GMZ2 administration to *Saimiri sciureus* induces high level of anti-GMZ2 antibodies as well (see below).

Functionality

Purified antibodies against GLURP and MSP3 have no significant direct growth inhibitory effect in *P. falciparum in vitro* cultures. However, when antibody preparations or plasma are co-incubated with monocytes and asexual blood stage *P. falciparum* cultures, growth inhibition can be measured (ADCI) *in vitro* and upon passive transfer *in vivo* [39]. Immunization of mice with GMZ2 induces growth-inhibitory antibodies in ADCI [28]. The same holds true when serum from GMZ2-vaccinated, malaria-naïve volunteers is used [37].

Safety, immunogenicity and efficacy in *Saimiri* monkeys

GMZ2 adjuvanted with aluminum hydroxide (alum), Montanide ISA720, or Freund's adjuvant was tested in *Saimiri sciureus* monkeys to assess toxicity, immunogenicity and efficacy upon subsequent challenge [40]. Vaccine preparations were injected subcutaneously three times. Animals receiving GMZ2 in Freund's adjuvant, got the first dose in complete Freund's adjuvant and the two subsquent doses in incomplete Freund's adjuvant. Local reactogenicity was low in the alum and Montanide groups, whereas animal receiving GMZ2 or control proteins (*L. lactis* culture supernatant) in Freund's adjuvant developed local inflammatory reaction with swelling at the injection site, which increased with the booster injections. Weight and hematological measurements remained normal in all groups. Immunogenicity was assessed by ELISA against GMZ2, MSP3 and GLURP as well as by immunofluorescence antibody test using fixed schizont stage parasites. GMZ2 in Freund's adjuvant was most immunogenic followed by Montanide (moderate and variable) and alum (low). Upon challenge by intravenous (IV) injection of asexual

blood stage *P. falciparum*, partial protection was observed in 0/5, 2/5 and 4/5 animals of the alum, Montanide and Freund's adjuvant groups. Sterile protection was not observed [40].

6.5.3. Clinical studies

So far, GMZ2 was clinically developed as an alum-adjuvanted vaccine for use in African children. A first-in-man study in Tübingen, Germany (ClinicalTrials.gov Identifier NCT00397449) was followed by three clinical trials in Africa: 1) 100 µg GMZ2 in alum against control (Rabies vaccine) in malaria-experienced adults from Lambaréné, Gabon (NCT00424944), 2) 30 or 100 µg GMZ2 in alum against control (Rabies) in 1–5 year old children from Lambaréné (NCT00703066) and 3) 100 µg GMZ2 in alum against control (Rabies) in 1–5 year old children from Gabon, Burkina Faso, Uganda and Ghana (Pan African Clinical Trials Registry trial number, ATMR2010060002033537). All studies assessed safety, tolerability and immunogenicity. The last trial is currently closing and has efficacy endpoints.

Assessment of the Safety and Immunogenicity of the Recombinant *Lactococcus lactis* Hybrid GMZ2 [GLURP+MSP3] a Malaria Vaccine in Healthy Adult Volunteers: A Phase 1, Randomised, Open, Dose-selection, Unicentre trial – GMZ2_1_04 (ClinicalTrials.gov ID NCT00397449)

The first-in-man phase 1 trial of alum-adjuvanted GMZ2 was a randomized, open-labeled, dose-escalating study [38]. It was conducted at the Institute of Tropical Medicine in Tübingen, Germany between Oct 2006 and December 2007. Malaria-naïve German adult volunteers received subcutaneous three injections of 10 µg (n = 10), 30 µg (n = 10) or 100 µg (n = 10) of GMZ2 four weeks apart (Day 0, 28 and 56).

Safety and tolerability of GMZ2 was excellent. Most adverse events (AE) were local. Six out of 30 volunteers had grade 3 local AEs (erythema and induration). All systemic AEs were mild or moderate. No GMZ2-related SAE occurred.

All three doses led to an increase in anti-GMZ2, -MSP3 and -GLURP antibody concentration and GMZ2-specific memory B-cells. Antibody concentration peaked at four weeks after the last immunization and remained high over the one-year follow-up. The highest dose (100 µg) induced higher anti-GMZ2 antibody levels after one injection but otherwise immune responses were similar between the groups. Anti-GLURP was generally higher than anti-MSP3 antibody concentration and the dominating IgG subclass was IgG1.

Since safety, tolerability and immunogenicity was shown, clinical development moved forward to a first-in-Africa study in healthy, male, adult participants with life-long exposure to malaria in Gabon.

A single centre, randomised controlled trial to evaluate the Safety and Immunogenicity of recombinant *Lactococcus lactis* hybrid GMZ2 [GLURP + MSP 3] blood stage malaria vaccine versus rabies vaccine in healthy Gabonese adult Volunteers – GMZ2_2_07 (ClinicalTrials.gov ID NCT00424944)

This randomized controlled, double-blind phase 1 trial took place at the Medical Research Unit in Lambaréné, Gabon between July 2007 and August 2008 [41]. Forty adults were randomly assigned to receive either 100 µg GMZ2 (n = 20) or control vaccine (n = 20) subcutaneously as three injections, each four weeks apart.

All 20 participants were administered GMZ2 whereas only 19 out of 20 participants were vaccinated with the rabies vaccine (one participant in the control group discontinued after the second vaccination for reasons not related to the study).

The vaccine was safe and well tolerated. No obvious difference in the AE pattern to the control vaccine was noted except for pain at injection site, which was more frequent in the GMZ2 group. All at least possibly related AEs were mild or moderate.

As expected, naturally acquired antibodies against the vaccine antigens were present before vaccination. GMZ2-specific antibody concentration increased following vaccination with GMZ2,

whereas controls remained unchanged. As in GMZ2_1_04, GLURP was the dominant immunogen. GMZ2-specific memory B-cells were higher in GMZ2 versus control vaccinees. One year after first vaccination antibody and memory B-cell levels returned to pre-vaccination values.
GMZ2_2_07 confirmed the results of GMZ2_1_04 and did not reveal an additional safety or tolerability signal. Due to the high natural pre-exposure to the vaccine antigens it was surprising that a booster effect on antibody and memory B-cell responses could be detected.

A phase I, randomized, controlled, double-blind, single centre trial to evaluate the safety and immunogenicity of 30 and 100 µg of GMZ2 in Gabonese children aged 1-5 years – GMZ2_3_08 (ClinicalTrials.gov ID NCT00703066)
This randomized controlled, double-blind phase 1 trial was performed in Lambaréné, Gabon from September 2008 until October 2009 to assess safety, tolerability and immunogenicity in the target group [42]. Thirty healthy children between one and five years of age, were randomly allocated to receive three injections of a control vaccine (rabies), 30 µg GMZ2 or 100 µg GMZ2 in the same schedule as in GMZ2_1_04 and GMZ2_2_07 (three injections, each injection 4 weeks apart). All injections were given intramuscularly (not subcutaneous as in the preceding trials).
As in the previous trial, GMZ2 was safe and well tolerated. No GMZ2-related serious adverse events (SAE) or local grade 3 AEs occurred. The AE pattern was similar between the three groups.
Antibodies and memory B-cell against GMZ2 developed in both GMZ2-vaccinated groups. Responses were not obviously dose-dependent and returned to very low levels one year after the first injection. A tendency towards an increased response against MSP3 was observed in the 100 µg GMZ2 group was present.
Since safety and tolerability were similar between the groups, it was decided to advance into a phase 2 trial with the 100 µg GMZ2 regimen.

A phase IIb, randomized, controlled, double-blind, multi-centre study to evaluate the efficacy, safety, and immunogenicity of three (3) doses of GMZ2 candidate malaria vaccine in Gabonese, Burkinabe, Ghanaian and Ugandan children aged 12 – 60 months – GMZ2_4_10 (PACTR registration number: ATMR201006000203353 7)
This multi-center randomized controlled, double blind phase IIb trial took place in four different African sites. The participants were randomly allocated in a 1:1 ratio to receive three intramuscular injections of a control vaccine (rabies) or 100 µg GMZ2 using the same regimen as in all previous GMZ2 trials (Day 0, 28 and 56). Preliminary analyses show that from 2366 screened children 1849 were randomized and 874 received all three injections of GMZ2. More than 1000 malaria episodes occurred within six months after the last vaccine injection. Hence, the study is well powered for efficacy analysis. In a preliminary analysis a ~10% vaccine efficacy was calculated and the vaccine was well tolerated and safe. All SAEs were judged not related to the intervention. Currently, data entry is ongoing. A final statistical analysis will be done upon completion of data entry. Reporting of results is scheduled for January 2015.

6.4.2 Adjuvant selection

6.4.2.1 Aluminum hydroxide
Aluminum hydroxide is one of the most commonly used adjuvants and has an extensive track record as a safe adjuvant, although alum-adjuvanted vaccines can cause local reactions of varying severity. It boosts immune responses against subunit vaccines and is in use since over 80 years [43, 44]. GMZ2-alum was extensively tested in phase 1 and 2 trials (see above) [38, 41, 42], where it was well tolerated, safe and immunogenic.

Preliminary analysis of the phase 2 trial on GMZ2-alum indicates that high antibody titers are required for protection, hence a formulation with a more potent adjuvant shall be used for further clinical development of GMZ2.

6.4.2.2 CAF01

Cationic Adjuvant Formulation 01 (CAF01) is an adjuvant system that was developed as part of a program to improve vaccines against tuberculosis [45]. It consists of dimethyldioctadecylammonium (DDA) cationic liposomes, combined with α,α'-trehalose 6,6'-dibehenate (TDB), a synthetic analog of trehalose 6,6'-dimycolate (TDM or cord-factor). It has potent immune-enhancing properties on humoral and cellular immune responses. Besides tuberculosis vaccines, it was tested with vaccine antigens for several other diseases [46] including malaria ($MSP1_{1-19}$), where it showed improved immunogenicity and protection over alum upon blood stage challenge in mice [47].

CAF01-adjuvanted vaccines for several diseases are under active clinical development. For human immunodeficiency virus (HIV), a peptide cocktail as therapeutic vaccine was tested in HIV-positive, treatment-naïve HIV infected patients in Denmark [48] and Guinea Bissau [49]. Both phase 1 trials show that the CAF01-adjuvanted vaccine is safe, well tolerated and directs immune responses towards the vaccinated peptides. A significant effect on viral load was not observed. In addition, the tuberculosis vaccine candidate Ag85B-ESAT-6 is currently tested with increasing doses of CAF01 (ClinicalTrials.gov ID NCT00922363), where it shows good safety and tolerability as well as long-lasting immunogenicity [50].

6.5.4. Microbial challenge studies in human volunteers

The deliberate infection of human volunteers with microorganisms has contributed uniquely to our understanding of the pathogenesis, immune responses and the treatment and prevention of numerous microbial diseases including influenza, cholera, typhoid fever and hepatitis [51]. A review by the UK Academy of Medical Sciences on microbial challenge studies recognized that such studies are important for providing proof of concept for therapeutic interventions and can significantly accelerate progress to Phase III studies [51]. GMZ2CAF01 will use controlled human malaria infection (CHMI) with IV administered PfSPZ Challenge (see below) to assess the efficacy of immunization with GMZ2.

6.5.5. Controlled human malaria infection

P. falciparum is a microbe particularly well suited to challenge studies. It has a relatively short asymptomatic, incubation period, a well-established diagnostic laboratory test (thick film microscopy), availability of highly efficacious chemotherapeutics, and no long term sequelae or infectious state following appropriate and timely treatment. Studies involving controlled human malaria infection (CHMI) are a powerful tool for investigating malaria vaccine and prophylactic drug efficacy [52]. With an increasing number of candidate malaria vaccines being developed, the number of centers conducting CHMI is expanding to increase the testing capacity worldwide [52].

Deliberate infection of humans with malaria was first performed in 1917 by Julius Wagner-Jauregg, primarily as a therapy for patients with neurosyphilis [53]. Thousands of patients underwent the treatment (the objective of which was to induce a febrile illness that was thought beneficial for the progress of the disease), administered by the bites of infectious mosquitoes or by intravenous or subcutaneous inoculation of dissected *Plasmodium* sporozoites suspended in media. The practice stopped with the advent of antibiotics.

In the 1960s, CHMI trials were used to assess the effects of anti-malaria treatments on healthy non-immune male inmates in the United States [54]. Following the development of protocols for the continuous culture of *P. falciparum* in 1976 [55] and for the generation of mature *P. falciparum* gametocytes *in vitro* in 1981 [56], it became possible to produce laboratory-reared infectious mosquitoes [57], meaning that CHMI trials could be performed more routinely.

The first well-documented CHMI with laboratory-reared infectious mosquitoes was carried out in 1986 at the US Walter Reed Army Institute of Research (WRAIR), the US Naval Medical Research Institute (NMRI) and the US National Institutes of Health (NIH). Six volunteers were infected with *P. falciparum* sporozoites by the bites of infectious *Anopheles freeborni* and *Anopheles stephensi* mosquitoes [58]. The following year, the efficacies of the first recombinant protein and synthetic peptide *P. falciparum* vaccines were tested in experimentally infected volunteers [59, 60].
CHMI has now become established as a key tool to assess the efficacy of novel malaria vaccines and drugs [52]. As CHMI trials are carried out in a controlled environment, they allow unprecedented detailed evaluation of parasite growth and immunological responses, providing essential information for vaccine and drug development [52].
Since the late 1980s, the number of institutions carrying out CHMI with *P. falciparum* has been growing. In 2007, data were published from a total of 532 volunteers [61]. Another report shows that a total of 1,343 volunteers were experimentally infected with *P. falciparum* between 1985 and 2009 [62].

6.5.6. CHMI with PfSPZ Challenge

In principle, the most accurate and practical way of dosing sporozoites is to inject them directly by needle and syringe [63]. Sanaria Inc. is a biotechnology company that has developed aseptic, purified cryopreserved PfSPZ for CHMI via injection (PfSPZ Challenge). The salivary glands of aseptic *A. stephensi* mosquitoes infected with PfSPZ are removed by dissection and triturated to release the sporozoites. The sporozoites are purified, counted and cryopreserved at a specified concentration to produce the inoculum, which is called PfSPZ Challenge. This process is in compliance with current Good Manufacturing Practices (cGMPs) and regulatory requirements for production of a high-quality PfSPZ Challenge product. Several studies to optimize dose and route of injection of PfSPZ Challenge were performed. The current standard for reproducible CHMI studies using PfSPZ Challenge is direct venous inoculation (DVI) of 3200 sporozoites. Intravenously administered PfSPZ Challenge have been used at three trial centers (Tübingen, Barcelona, CERMEL) so far and successfully infected all malaria-naïve volunteers (n = 53) and at least 60% of highly exposed semi-immune Gabonese volunteers. In total 73 volunteers received CHMI by intravenous injection of PfSPZ Challenge, so far (NCT02115516, NCT02237586, NCT01624961, NCT01771848). The product has been well tolerated and safe. Volunteers who became slide positive experienced malaria symptoms.

6.5.7. Conduct of CHMI trials

Following a collaborative consensus process involving investigators from the USMMVP, Sanaria Inc., University of Maryland, University of Oxford, RUNMC, The Seattle Biomedical Research Institute and the KEMRI-Wellcome Kilifi Research Programme, a consensus document; 'Standardization of Design and Conduct of *P. falciparum* Sporozoite Challenge Trials' was developed, and provides a comprehensive guide to the appropriate conduct of malaria challenge studies [64, 65]. Although there remain minor differences between centers in follow-up procedures in CHMI trial conduct, there is consensus on the following key points:

- All volunteers should have a medical assessment no longer than 48 hours before challenge, including an interim medical history, directed physical examination, and pregnancy test for female volunteers.
- Follow-up visits should be scheduled at least once daily, but may increase in frequency to two or three times daily, starting between Day 5 and 7 post-challenge. At all visits volunteers should be questioned about the occurrence of AEs and use of medication.
- In the event that a volunteer does not attend for a scheduled follow-up visit it is imperative that investigators find the volunteer as quickly as possible and assess for patent parasitemia and clinical malaria. Should the volunteer withdraw consent from further follow-up prior to receipt of antimalarial drugs, it may be appropriate to administer a course of antimalarial chemotherapy under close supervision.

- Grading and reporting of AEs should be performed using international and local guidelines. It should be noted that the occurrence of a low frequency of Grade 3 AEs, of short duration and with no long-term sequelae, is not unexpected in clinical CHMI. A minority of those challenged is known to experience Grade 3 systemic AEs and this fact should be included in the informed consent form.
- Vital signs should be recorded at least once daily and at any subsequent visits for medical attention. Directed physical examination should be performed when necessary.
- It is critical that every volunteer must receive every dose of antimalarial therapy. In some settings, fully directly observed treatment will be essential. Where directly observed treatment is not used, investigators must follow volunteers closely to ensure compliance with the treatment regimen.
- After challenge, all volunteers should be followed until they have completely finished antimalarial treatment.
- Volunteers should be evaluated at least two weeks after finishing treatment.
- A local safety monitor and an independent SMC should be established to act as independent experts in evaluating AEs. The safety monitor or monitoring committee may advise the investigators on initiating antimalarial treatment for a specific volunteer or volunteer group. While SMCs are not a requirement for Phase 1 trials, they should be considered a requirement for malaria challenge trials which have an efficacy component and which have major potential safety concerns.

6.5.8. Clinical presentation under CHMI

Nearly all malaria-naïve volunteers in CHMI studies develop symptoms of malaria; approximately one-fifth of volunteers temporarily develop symptoms graded severe (symptoms that prevent daily activities) but serious or life-threatening malaria has never occurred [66]. The most common symptoms are fatigue and headache, and severe symptoms can include headache, fatigue, malaise, chills, myalgia, rigors, nausea and vomiting. Clinical symptoms generally coincide with the detection by microscopy of thick film blood smears of blood-stage parasites at densities of 10 to 20 parasites per µL of blood [66]. This corresponds to a parasitemia of approximately 0.0004% infected erythrocytes [63]. Severe malaria is generally diagnosed when parasitemia is at least 1000-fold higher than the peak parasitemia in CHMI trials. After the start of antimalarial treatment, symptoms can temporarily increase in severity but subside quickly within approximately 2 to 3 days [63]. Routine laboratory checks generally show a moderate decrease in leucocyte and platelet numbers during infection, with no change in hemoglobin concentration [67]. Bleeding or thrombogenic complications have never been described [66, 67]. Abnormalities of liver enzymes have been observed, but these abnormalities did not result in clinical manifestations and they resolved after a few days to weeks [66, 67].

Immediate treatment of volunteers at the earliest phase of microscopically detectable blood-stage infection ensures that the potential risks of complications associated with severe malaria are minimized to the greatest extent possible. Indeed, CHMIs have been shown to be safe in all volunteers treated since 1985 when the first volunteers were infected by exposure to mosquitoes that had fed on cultures containing *P. falciparum* gametocytes [65–69].

Several years ago, safety concerns were raised because of a cardiac event in a young volunteer who underwent CHMI by mosquito bite at Radboud University Nijmegen Medical Center (RUNMC) in the Netherlands shortly after initiation of antimalarial treatment for Pf infection. The adverse event resolved following treatment and did not re-occur in the one-year follow-up period. A definite relationship between the cardiac event and the experimental infection or its treatment was not established [70]. In addition, there was a cardiac related SAE in an ongoing phase I trial in the Netherlands where PfSPZ Challenge has been administered to volunteers in combination with the antimalarial drug, chloroquine (TIP5, ClinicalTrials.gvo ID: NCT01728701, see above). Here, one subject experienced an episode of myocarditis (serious adverse event)

following treatment for malaria. Specifically, the SAE occurred in Volunteer 1641-27, who underwent CHMI on Day 124 using five Pf NF54-infected mosquitoes. The CHMI occurred 60 days after he had received the last injection of PfSPZ Challenge for immunization. On Day 9 post-CHMI the volunteer had a sore throat. On Day 11 post-CHMI (16th February 2013), the volunteer's thick film was positive for malaria, and treatment with atovaquone-proguanil was initiated. The subject was asymptomatic, but due to elevated Troponin T levels (maximum: 299 ng/L), which are routinely assessed at RUNMC on a daily basis, the volunteer was hospitalized on 18th February 2013 (Day 13 after CHMI). On 19th February 2013 the volunteer experienced chest pain for 10 minutes and received sublingual nitroglycerin spray. He was diagnosed with myocarditis based on a magnetic resonance imaging (MRI) and minor repolarization disturbances on electrocardiogram. The volunteer had no further symptoms on follow-up, and the Troponin T levels returned to normal by 5th March 2013. The volunteer was discharged on 22nd February 2013, and the electrocardiogram was normal on 8th March 2013. A follow-up cardiac MRI (8th July 2013), performed approximately 5 months after the start of the SAE, demonstrated good left ventricular function with mild hypokinesia in a few segments and some remaining mid-wall delayed enhancement. An etiology was not established, but the volunteer's throat swab was positive for rhinovirus on 19th February 2013. Furthermore, 14 days after the third immunization with PfSPZ Challenge, the volunteer received immunizations against six pathogens in preparation for travel. Nevertheless, it has been generally agreed that volunteers with an increased risk of cardiac disease should be excluded from such trials [64]. The spectrum of adverse events induced by PfSPZ Challenge CHMI is very similar to CHMI using infectious mosquitoes or injection of blood-stage parasites.

6.5.9. Ethical considerations of CHMI trials

For any clinical trial, the risk for potential volunteers should be compared with the benefit. As no direct benefit accrues to CHMI trial participants, indirect benefits are considered in the context of possible public health gains that may occur as a result of scientific advances made through CHMI. This places a burden on the CHMI trial investigators both to exercise all possible safeguards for volunteer safety (primary consideration) and to ensure that maximal scientific benefit accrues from each CHMI trial (secondary consideration). Key ethical considerations agreed by consensus of the field [64] include;

- Safety is the paramount consideration in conduct of CHMI trials. When CHMI trials are conducted at existing and new centers, volunteer safety is the main focus of practical considerations.
- Investigators are required to follow both international and local guidelines with respect to ethical considerations and in accordance with the Declaration of Helsinki and should fulfill all local regulatory and ethics committee requirements.
- CHMI trials should be conducted according to Good Clinical Practice Guidelines (either ICH or WHO). The scientific benefit should be maximized whilst minimizing risk, discomfort or distress to individuals. From this perspective it is important that the results of CHMI trials enable comparative evaluation and collection of as much information as is reasonably possible, both in terms of the load of merozoites that emerges from the liver (the "liver-to-blood inoculum") and the blood-stage parasite growth rate prior to initiation of drug treatment as well as the proportion of volunteers completely protected. The availability of data to the scientific community also attains an ethical dimension in this perspective, with importance attached to access to data that may inform design of future CHMI trials and design of malaria vaccines.
- The raw data (both microscopy and PCR where available) from CHMI trial datasets should be made publicly available to facilitate scientific benefit to the community.
- If an unexpected SAE, which is possibly related to CHMI occurs at a CHMI trial centre, recognizing legal restrictions, every effort should be made to communicate information

on this SAE to the community of CHMI trial centers within 90 days of the occurrence of the SAE. This is in addition to the usual reporting requirements to ethical committees, sponsors and regulatory authorities. SAEs that are unambiguously not related to the challenge procedure are excluded, e.g. hospitalizations for clearly coincidental events such as trauma. Where there is any doubt community-wide notification should occur. This is because safety of participants at other centers may be affected by occurrence of an SAE at one center.

6.6. Summary of known and potential risks and benefits to human subjects

6.6.1. Potential risks

Investigational product

So far GMZ2 was given to a limited number of volunteers. Safety and tolerability was excellent so far but due to the restricted sample size it cannot be ensured that no rare serious reactions occur. Injection of alum-adjuvanted GMZ2 frequently leads to mild (grade 1) local reactions. Occasionally, moderate (grade 2) and severe (grade 3) local reactions occur. All reported adverse reactions were reversible, usually within 24 hours, and did not require treatment. CAF01-adjuvanted vaccines have been administered to >50 participants. Safety and tolerability was good with few reported low-grade local reactions and rare low-grade systemic AEs. The number of exposed individuals is still low but includes African study participants. As expected, no different pattern to Europeans was observed. Formulation of GMZ2 with CAF01 will be assessed for the first time in the current trial. Although unlikely, serious local and systemic reactions may occur. To minimize risk to the participants, all injections will be done in a clinical area by clinicians trained in advanced life support (ALS). Injections will be done in small sentinel groups first and all participants are required to stay in the unit for at least one hour following vaccination.

Phlebotomy

The maximum volume of blood drawn over the study period (≤ 500 ml over a 8 month period) is an accepted standard for healthy individuals that should not compromise the donor and is reflected in the German guidelines for blood donation [71]. According to these guidelines, the total blood volume sampled should not exceed 2000 mL (3000 mL for men) within 12 months and single donations should not exceed 500 ml. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venipuncture, which will not be documented as AEs if they occur. In rare cases arteries or nervous tissue may be injured or a punctured vessel may occlude and induce inflammation of the surrounding tissue.

***Plasmodium falciparum* infection**

After inoculation with PfSPZ Challenge for CHMI, those not protected by GMZ2 are likely to develop malaria. Symptoms and signs of malaria may include fever, tachycardia, hypotension, chills, rigors, sweats, headache, anorexia, nausea, vomiting, diarrhea, myalgia, arthralgia, low back pain, thrombocytopenia, and lymphopenia. Thirty to 50 percent of malaria-naïve volunteers are likely to experience at least one severe (Grade 3) adverse event related to Pf infection [72]. Unmonitored and untreated, Pf infection can be fatal – however – only after several additional replication cycles of 48 hours each after the onset of symptoms. For this reason, volunteers will be enrolled in the study only if they are deemed reliable and capable of complying with the follow-up schedule. If necessary, participants can be admitted for in-patient care at the Albert Schweitzer Hospital. Since all participants are semi-immune and have naturally acquired many *P. falciparum* infections, it is highly unlikely that complications occur. Participants will be followed up to 35 day after PfSPZ Challenge injection. In case that low-grade parasitemia but no symptoms develop, treatment will be postponed until symptoms occur, the parasitemia is ≥ 1000 parasites per microliter or Day 35 is reached.

Treatment of *Plasmodium falciparum* infection

After CHMI, volunteers will be treated with a registered, oral, proven, and highly efficacious treatment under supervision of a study team member. First-line treatment will be artemether-lumefantrine. Since the PfSPZ Challenge parasite strain NF54 is sensitive to all currently registered antimalarial drugs any antimalarial that is registered for the treatment of Pf infection can be used as second line treatment. No case of drug-failure has been observed in the more than 1,343 documented volunteers infected between 1985 and the present. Most trials have used the Pf 3D7 strain, a clone of the Pf NF54 strain that is contained in PfSPZ Challenge.

Despite the fact that all well documented volunteers since 1985 were successfully treated, a small probability of drug failure remains (for 1,343 successfully treated volunteers the 97.5% binomial exact confidence interval is 0 to 0.27%). In case of drug failure or inability to receive oral medication, an alternative parental treatment will be given according to the National guidelines for the treatment of malaria.

6.6.2. Potential benefits

Participants will not benefit directly from participation in this study. Information about their general health status may be a potential indirect benefit. However, it is hoped that the information gained from this study will contribute to the development of a safe and effective antimalarial intervention. Improving efficacy of GMZ2 will help to develop better malaria control measures. Besides protecting individuals from malaria, asexual blood stage malaria vaccines such as GMZ2 can complement for the loss of semi-immunity that may occur upon implementation of highly efficacious interventions in the framework of malaria elimination campaigns.

6.5 Rationale for conducting the trial

GMZ2 is an asexual blood stage candidate, designed to mimic naturally acquired immunity. Only few blood stage malaria vaccine candidates were clinically developed until efficacy was tested and those that reached this level did not show a clinically significant effect. Following a phase 1 program, alum-adjuvanted GMZ2 was tested in large clinical trial in four centers scattered over Africa (West, Central and East Africa). Preliminary data indicate that GMZ2 is the first asexual blood stage malaria vaccine candidate that mediates protection from malaria. The level of protection is low and data suggest that enhancing immunogenicity shall improve efficacy significantly. Introduction of new adjuvant systems can be critical, as it has been in the case of RTS,S [73], the only malaria vaccine candidate that was tested in a phase 3 clinical trial and which will be licensed soon.

6.5.2 Rationale of GMZ2-alum and GMZ2-CAF01 dosing and schedule

Most data exist on 100 µg GMZ2 adjuvanted with alum. The dose shows consistent immunogenicity in malaria-naïve and -exposed study participants and is safe and well tolerated. Hence the target dose of CAF01 formulated GMZ2 is 100 µg. In order to assess if dose-sparing is possible and as a sentinel group, 30 µg CAF01-adjuvantes GMZ2 will be assessed first. Since maximum humoral immune response against GMZ2 is the aim of the study no further dose de-escalation will be done.

7. Study objectives and endpoints

7.1. Objectives

Primary

Establish a regimen of GMZ2-CAF01, which is safe and well tolerated.

To assess safety and tolerability, AEs will be collected, graded and related to vaccination by evaluation of causality [74].

Secondary
Establish a regimen of GMZ2-CAF01 that reduces parasite multiplication upon controlled human malaria infection (CHMI)
Time to malaria, parasitemia and parasite kinetics over time will be measured following DVI of PfSPZ Challenge using clinical assessment, thick blood smear microscopy and quantitative polymerase chain reaction (qPCR), respectively.

Establish a regimen of GMZ2-CAF01 that induces a superior anti-GMZ2 antibody-response compared to GMZ2-alum and a control vaccine (Rabies).
Anti-GMZ2 antibodies during GMZ2-CAF01 vaccination will be measured by ELISA over time and compared between the groups.

Exploratory
Assess functional activity of vaccine-induced antibodies.
Increase in functional activity of vaccine-induced antibodies will be assessed by ADCI and antibody-dependent respiratory burst (ADRB) assay [75]. Additional exploratory analyses will be done to characterize type and longevity of vaccine-induced immune responses as well as genetic diversity in immune responses.
Assess the effect of microbial composition of blood, stool and urine on vaccine-induced immune responses.
Assess the effect of metabolites in blood and urine, measured by non-biased methods, on vaccine-induced immune responses.

7.2. Endpoints

Primary safety endpoint
Number or occurrence of at least possibly related Grade 2 AEs, Grade 3 AEs and SAEs from time of first vaccine injection to the end of the follow-up period.

Secondary safety endpoint
Number of at least possibly related local, systemic and unsolicited AEs from time of first vaccine injection to the end of the follow-up period. This definition includes all grade 1 AEs and laboratory abnormalities.

Primary efficacy endpoint
Time to malaria from DVI of PfSPZ Challenge.

Secondary efficacy endpoint
Time to asexual blood stage parasitemia from DVI of PfSPZ Challenge.

Primary immunogenicity endpoint
Difference in baseline-corrected area under the curve (AUC) anti-GMZ2 antibody concentration between groups.

Exploratory immunogenicity endpoints
- Baseline-corrected anti-MSP3, -GLURP and -GMZ2 antibody concentration four weeks after third vaccine injection.
- Relative concentration of anti-MSP3, -GLURP and -GMZ2 IgG subclasses (IgG1–4) four weeks after third vaccine injection.
- Baseline-corrected growth inhibition in ADCI
- Baseline-corrected induction of ADRB

- Number and phenotype of GMZ2-reactive T helper cells
- Increase in GMZ2-specific memory B-cells
- Increase in circulating plasmablast
- Induction of cross-reactive antibodies
- Genetic variation in immune genes (e.g. major histocompatibility and antibody receptor genes)
- Composition of urine and stool microbiota
- Metabolite pattern in urine and blood

8. Study design and site description

8.1. Study design

This study is as a randomized, controlled, double-blinded, single center phase 1 trial with five arms:

A: Control vaccine (Rabies); n = 8

B: 100 µg of the GMZ2-alum; n = 12

C: 30 µg of the GMZ2-CAF01; n = 8

D: 100 µg of the GMZ2-CAF01; n = 12

E: 100 µg of the GMZ2-CAF01 without subsequent CHMI; n = 10

All injections will be administered intramuscularly (IM) in the deltoid muscle. Injections will be given at alternating sides on Days 0, 28 and 56. Follow-up will be done over six months after the last vaccination. Total duration of the trial will be 12 months.

Participants will be vaccinated in four time slots. First injections will be done on:

Study day 0: A (n = 2), B (n = 3), C (n = 1)
Study day 1: A (n = 2), B (n = 3), C (n = 5), D (n = 1)
Study day 2: A (n = 2), B (n = 3), C (n = 1), D (n = 5), E (n = 5)
Study day 3: A (n = 2), B (n = 3), C (n = 1), D (n = 6), E (n = 5)

Five to eighteen weeks after completion of the full schedule of three vaccine injections CHMI by DVI of 3200 PfSPZ Challenge will be done in participants of Groups A, B, C and D. Group E will not receive CHMI and serve as a safety control group for the new GMZ2-CAF-01 vaccine.

8.2. Study site

The study will be conducted at the Centre de Recherches Médicales de Lambaréné (CERMEL), Gabon. CERMEL emanated out of the Medical Research Unit of the Albert Schweitzer Hospital, which was established in 1981 and is an integral part of the Albert Schweitzer Hospital. CERMEL has an extensive track record on clinical trials (www.cermel.org), performed according to "International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice (ICH-GCP)" guidelines. Amongst others, CERMEL was involved in three GMZ2 trials (phase 1 and 2), the phase 2 and 3 program of RTS,S, a challenge study with PfSPZ Challenge and phase 1, 2 and 3 trials on antimalarial drugs.
Other areas of research include diagnostics, immunology, vaccinology, schistosomiasis, filariasis and mechanisms of allergy development. The United States' Food and Drug Agency (FDA) repeatedly audited the center.

Infrastructure, trained personnel and procedures to conduct phase 1 trials are on site. This includes dedicated study areas with advanced life support equipment and immediate access to the hospital infrastructure. Ethical and regulatory review exists on the national level. An institutional scientific and ethics review committee is present.
A multi-disciplinary team has been set up to undertake GMZ2CAF01.

9. Selection of participants and contra-indication to vaccine administration

The study population is healthy Gabonese adults living in Lambaréné (Gabon) with a life-long exposure to malaria.

9.1. Inclusion criteria

The participant must satisfy all the following criteria to be eligible for the study:

- Healthy adults aged 18 to 40 years.
- Able and willing (in the Investigator's opinion) to comply with all study requirements.
- Agreement to refrain from blood donation during the course of the study and after the end of their involvement in the study according to the local blood banking eligibility criteria
- Residence in Lambaréné or surroundings for the period of the trial.
- History of long term residence (>10 years) in area known to have significant transmission of *P. falciparum*
- Written informed consent to receive GMZ2 for immunization and PfSPZ Challenge for CHMI.
- Answer all questions on the informed consent quiz correctly.
- Willingness to take two curative anti-malarial regimens.
- Reachable (24/7) by mobile phone during the immunization, CHMI and follow-up.
- A body mass index <35.

9.2. Exclusion criteria

The participant may not enter the study if any of the following apply:

- Receipt of an investigational product in the 30 days preceding enrollment, or planned receipt during the study period.
- Prior receipt of an investigational malaria vaccine.
- Immunization with more than 3 other vaccines within the past month.
- Positive HIV test.
- Any confirmed or suspected immunosuppressive or immunodeficient state, asplenia, recurrent, severe and chronic (more than 14 days) infections, immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed).
- Use of immunoglobulins or blood products within 3 months prior to enrolment.
- Sickle cell disease or any clinically relevant blood disorder.
- A history of allergic disease or reactions likely to be exacerbated by vaccine administration.
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma *in situ*).
- History of serious psychiatric condition that may affect participation in the study.
- Any other serious chronic illness requiring hospital specialist supervision.

- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 60 g per day.
- Suspected or known injecting drug abuse in the 5 years preceding enrollment.
- Contraindications to the use of the first-line anti-malarial medications: artemether/lumefantrine or atovaquone/proguanil.
- Positive for hepatitis B surface antigen (HBs-antigen).
- Seropositive for hepatitis C virus (antibodies to HCV).
- Subjects unable to be closely followed for social, geographic or psychological reasons.
- Any clinically significant abnormal finding on biochemistry or hematology blood tests, urine analysis or clinical examination.
- History of seizure.
- Subjects unable to be closely followed for social, geographic or psychological reasons.
- Abnormal electrocardiogram on screening: pathologic Q wave and significant ST-T wave changes, left ventricular hypertrophy, non-sinus rhythm except isolated premature atrial contractions, right of left bundle branch block, advanced A-V heart block (secondary or tertiary).
- A QT/QTc interval > 450 ms.
- Any other significant disease, disorder or finding which, in the opinion of the Investigator, may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.

In case of inconclusive results of laboratory tests or other diagnostic procedures, tests will be repeated. If doubts about the results persist, volunteers will be considered ineligible.

9.3. Withdrawal

In accordance with the principles of the current 7th revision of the Declaration of Helsinki (updated 2013) [76] and any other applicable regulations, a study participant has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw a participant at any time in the interests of the participant's health and well-being. In addition, study participants may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator.
- Ineligibility (e. g. arising during the study).
- Significant protocol deviation.
- Participant non-compliance with study requirements.
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

The reason for withdrawal will be recorded on the CRF. If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the participant, until the AE has resolved, stabilized or a non-trial related causality has been assigned. Any participant who is withdrawn from the study may be replaced, if that is possible within the specified time frame. The Local Safety Monitor (LSM) may recommend withdrawal of participants.

If a participant withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored unless the participant specifically requests otherwise. All participants

can decide at any time if samples and data may be used later or will be permanently destroyed. This will be explained and documented in a separate data protection form.

9.4. Criteria to stop vaccination

If any of the following criteria become applicable during the trial, any further vaccination will not be administered but follow-up shall be continued.

- Acute allergic reaction (significant IgE-mediated events) or anaphylaxis following the administration of vaccine investigational product.
- Continuous significant illness (e.g. temperature >38°C) for more than 14 days following the scheduled day of vaccination.
- Use of any experimental medicine (drug or vaccine) other than the study vaccines or PfSPZ Challenge during the study period.
- Administration of immunoglobulins and/or any blood products during the study period.
- Chronic administration (more than 14 days) of immunosuppressants or other immune-modifying drugs during the study period (for corticosteroids: prednisone or equivalent ≥ 0.5 mg/kg/day. Inhaled and topical steroids are allowed).
- Administration of the study vaccine at incorrect route.

9.5. Criteria to delay vaccination and CHMI

The following AEs constitute contraindications to vaccine or PfSPZ Challenge administration at that point in time. If any one of these AEs occurs at the day scheduled for vaccination or PfSPZ Challenge inoculation, injection may be delayed for up to 14 days. The investigator may withdraw the participant at any time in case there is a significant increase in risk for the participant.

- Acute disease at the time of administration of investigational product (acute disease is defined as the presence of a moderate or severe illness with or without fever). All vaccines can be administered to persons with a minor illness such as diarrhea or mild upper respiratory infection without fever, i.e. axillary temperature < 38°C.
- Axillary temperature of > 38°C.

10. Study procedures

Before trial activities begin, formal meetings will be held with respective administrative, medical authorities and local leaders to explain the purpose of the trial.

10.1. Screening

Eligible screened participants will be included within a maximum of 21 days. Field worker will introduce potential participants to the trial at their homes and invite them to CERMEL for a more formal presentation in case they are interested. At CERMEL, potential participants will be interviewed and in case of continuing interest interviewed separately. Participants who give signed and witnessed informed consent will be screened for trial eligibility. Eligible participants will be enrolled in the trial and assigned a study identification number (ID). A screening log will be used to record name, screening number and outcome of the screening.

10.2. Randomization, vaccine allocation and enrolment

A computer-generated randomization list will be used to allocate participants to the intervention groups. Two randomization card sets will be distributed in sealed envelopes, marked with the study ID. The local safety monitor (LSM) and the formulation team will receive one set of sealed randomization cards shortly before first vaccine injection. During vaccine preparation, the copy of the formulation team will be used to prepare the vaccine. Participants may be replaced on the day of first vaccine injection in case they do not show up.
An enrolment log will be used to document enrolment and eventual replacements.

10.3. Vaccination process

The sponsor shall ensure that all vaccines and adjuvant as well as all supplies required for vaccination have been adequately received and appropriately stored at the site before initiation of the trial. Vaccines will be formulated on the respective vaccination days according to a standard operating procedure (SOP) by trained and qualified staff.

The vaccines will be given to participants at the study center within 21 days of assessment for eligibility. Qualified medically trained personnel will inject vaccine formulations. An ALS-trained clinician will be present during vaccinations. Vaccine allocation is concealed from the study team performing all subsequent manipulations. Vaccine injectors will not be informed about the formulation but appearance of the different formulations is different. Hence, they are not fully blinded and excluded from further responsibilities within the clinical trial until he blind is lifted.

Before each vaccination, in- and exclusion will be re-reviewed and verified by the investigator. A history-directed physical examination will be performed and temperature and baseline general symptoms will be recorded. Venous blood will be collected for laboratory analyses. After the participant's identity is checked the vaccine will be injected intramuscularly. The first and third injections will be given in the deltoid muscle of the left arm and the second injection in the right arm. If any local condition that prevents administration of the vaccine into the preferred deltoid for that particular immunization, the vaccine may be administered into the opposite deltoid. Vaccination will be performed on Days 0, 28 and 56. Maximal deviation from the intended second and third vaccine injection is 14 days. In case of delayed vaccine administration all subsequent procedure will be delayed. Hence, third vaccination has a maximal delay of 28 days.

Medication and equipment for the treatment of immediate adverse reactions, including anaphylactic shock will be reviewed on each vaccination-day before first vaccine injection.

10.4. Follow-up

After each vaccination, participants will be observed for solicited local and systemic adverse events for at least 30 minutes and shall not leave the clinical area before one hour after injection. Physicians or delegates trained in resuscitation will be present on site on vaccination days. Signs and symptoms will be solicited from participants at the end of the 30-minute period and recorded by the investigator or its designee. Field workers will follow participants on Days 2, 4 and 6 after each vaccination. They use open interviews and a structured form to capture any AE. Participants will be seen by the physician or designate on Days 1, 7 and 14 post-vaccination. Participants will be actively encouraged to report any AE during the study. A 24-hourly operated telephone line will be available to contact the study team at any time. At each visit on site, a study physician will evaluate the participants. A clinical examination will be performed and information about any solicited or unsolicited signs/symptoms since the last visit will be collected. Every effort will be made to ensure compliance with visits. Field workers will conduct home visits to participants a day before their scheduled visit at the Health Centre. If a participant does not appear for a scheduled clinic visit, the field worker will visit him/her again on the same day to uncover reasons for non-attendance. If a SAE occurs, appropriate measures will be taken to notify the PI, LSM, Sponsor, SMC, EC as per requirement. The total follow up duration will be six months after administration of the last dose of the vaccine with month visits after last vaccine injection.

10.5. Lost to Follow-up Procedures

If a participant fails to appear for a follow-up examination, intensive efforts to visit them at home will be undertaken to recall them and to determine their health status. These efforts will be documented in the participant's file at the site.

10.6. Controlled human malaria infection

Participants will receive a radical cure treatment for malaria with 300 mg clindamycin twice a day for five days, starting four weeks after the last vaccine injection. PfSPZ Challenge is injected

three days later. Participants remain at the site for one hour after inoculation in case that any immediate adverse events occur. They will be examined one day later and contacted daily by phone or a field worker's visit until Day 5 after PfSPZ injection. From Day 6 to Day 35 participants are seen daily to capture clinical signs and symptoms as well as for sampling of parasitemia. Maximal allowed time deviation from the last vaccination is a 14 days delay (total 6 weeks). In case of delayed PfSPZ Challenge administration all subsequent procedure will be delayed accordingly.

10.7. Malaria diagnosis and treatment following CHMI

Blood smears and a 1 ml sample for DNA extraction will be collected daily, approximately 24 hours apart. In the case of a symptomatic subject, blood smears may be collected as frequently as every 6 hours or at any time post-challenge, based upon the clinical judgment of the investigator. A thick film is considered positive if at least 2 unambiguous malaria parasite structures are seen in 0.5 µl of blood (4 parasites per µl). Positive smears should be confirmed by an expert malaria microscopist as well as by the clinician managing the subjects at the time the smear is read as positive.

P. falciparum infection will be treated with artemether-lumefantrine as the first line treatment. All drugs are given in doses and time intervals as per the package insert and recommendations of the authorities under supervision by an investigator. The NF54 parasites are known to be fully sensitive to both antimalarial regimens. Prior to starting antimalarial treatment participants will be screened for drug interactions and contraindications to the respective drug(s). Participants will be reminded of the potential side effects of the antimalarial and given the patient information sheet for their treatment regimen. The study personnel will observe treatment.

When a case of malaria is diagnosed, each subject will have a clinical evaluation by one of the investigators (a physician) with appropriate history and physical examination where deemed to be necessary. Intake of the antimalarial is observed and participants remain in the clinic for at least 30 mins after dosing. If necessary, they can be admitted to the clinical ward of CERMEL for observation and further medical management. A subject will only be considered cured of *P. falciparum* infection when 2 consecutive blood smears are negative.

The investigators are able to treat any participant for malaria regardless of the thick film microscopy or PCR result if they are clinically concerned (and have discussed the case with the Principal Investigator), or when a participant wishes to withdraw from the study.

10.7.1. Criteria for treatment

Treatment will be given according to the following guidelines:

- Under observation
- Immediately once thick blood smear is positive and symptoms that can be attributed to malaria are present
- A parasitemia >1000 parasites per µl, irrespective of symptoms
- On Day 35 of follow up if all blood smears taken up to Day 35 have been negative
- Based on recommendations of study physicians and Safety Monitoring Committee
- On the request of participants or withdrawal of consent

If a patient is unable to tolerate an oral anti-malarial, the participant will be admitted for inpatient care and an appropriate parenteral anti-malarial therapy prescribed.

If a participant withdraws/is withdrawn from the study after receiving PfSPZ Challenge but before reaching the criterion for malaria diagnosis, a complete, appropriate, curative course of anti-malarial therapy must be completed. The importance of this will be emphasised to volunteers at screening and during the consent process.

10.7.2. Malaria symptoms

Treatment is given upon positive blood smear and presence of malaria symptoms. Typical malaria symptoms include fever, tachycardia, chills, rigor, sweats, headache, anorexia, nausea, vomiting, myalgia, arthralgia, chest pain, low back pain, abdominal pain and fatigue.

10.7.3. Supportive medications

On development of symptoms, provided there are no contraindications, participants will be permitted to take over-the-counter antipyretic medication (e.g. ibuprofen).

10.7.4. Criteria for hospital admission

If any of the following criteria are met, hospital admission will be considered:

- Failure of symptoms to improve within 48 hours of starting anti-malarial therapy
- Unable to tolerate oral artemether/lumefantrine, atovaquone/proguanil, or alternative
- Dehydration requiring intravenous fluid therapy
- Signs or symptoms suggestive of pulmonary oedema
- Signs or symptoms of neurological dysfunction including altered consciousness
- Signs, symptoms or laboratory evidence of significant renal or hepatic dysfunction
- Unanticipated concern about subject's home circumstances
- Any other significant finding, which the Investigator feels warrant in-patient admission.

Ultimately, the decision regarding admission will be taken by the investigators in conjunction with the physician on call.

10.7.5. Follow-up post diagnosis

Participants will be reviewed in the clinic approximately 24 and 48 hours after diagnosis (and the start of anti-malarial therapy) when physical observations, symptom questionnaire and venepuncture for PCR and blood film will be performed. If blood films taken at 24 and 48 hour post diagnosis are negative for parasites and the patient is asymptomatic or has mild, resolving symptoms, the participant will not be seen again in clinic until Day C+35. Otherwise, the participants will continue to be reviewed in clinic daily until they have 2 consecutive negative blood films at least 24 hours apart following start of antimalarial treatment, and until all symptoms are mild or resolving. Participants will be called by telephone to document the end of any outstanding malaria symptoms on-going between completion of antimalarial treatment and Day C+35.

10.7.6. Safety measures for challenge

Participant safety is of paramount importance. The following measures are in place to safeguard participant safety during CHMI:

- Participants' understanding of the trial information will be tested by means of a questionnaire at screening. This provides further confidence that fully informed consent has been obtained.
- If the subject does not have their own mobile telephone they will be issued with one for the duration of the study and counselled about the importance of keeping it switched on or checking the messages regularly.
- Before challenge, full contact details for each subject will be documented, including home address and mobile telephone numbers. Mobile telephone numbers will be verified prior to challenge to ensure the participants are easily contactable. Home and work landline telephone numbers where available will be documented and next of kin identified. At least two emergency contact numbers will be confirmed and verified as authentic for each subject who will participate in the challenge. At least one of these emergency contacts

should be a close friend, relative or housemate who lives nearby and will be kept informed of their whereabouts for the duration of the study.

- Prior to challenge, the clinical study team will review subject adherence to the safety follow-up schedule to date. This review will attempt to identify any likelihood of unreliability on the part of the subject during the challenge phase of the study. In the event that a subject shows a pattern of missed follow-up visits, the clinical team will discuss and emphasize the importance of compliance with all follow-ups to ensure safety throughout the post-challenge period. Any subject expressing inability to comply with study requirements may be deemed unsuitable for challenge and will be excluded from the challenge phase of the study. All subjects who have received PfSPZ Challenge, however, will be encouraged to remain in the study for safety follow-ups.
- On the day of challenge, participants will be provided with a medic alert card containing contact details of the study team, brief details of the study and the drug sensitivities of PfSPZ Challenge.
- On days 2, 3 and 4 post challenge when the participants do not have scheduled clinic visits, participants will be visited or contacted daily by the clinic team in order to ensure they are well and contactable.
- Subjects will be counselled to use methods that will reduce risk of exposure to mosquitoes from day 5 until day 35 post-challenge or until the second negative smear after treatment. Recommendations will be provided on the day of challenge.
- Participants will be able to contact a medically qualified member of the study team 24 hours a day throughout the study period and will be instructed to contact the investigator immediately should any serious signs or symptoms occur.
- If necessary, the study team will visit participants at home if they are unable to attend clinic for review.
- The study team will observe administration of antimalarials.
- Participants will be counselled that failure to return for treatment after having been infected with *P. falciparum* may result in becoming very unwell and potentially die. They will be instructed to remain in Lambaréné and the immediate surrounding area for the duration of the intensive follow-up schedule (days 1-35 post challenge). They will be informed that should they fail to attend a scheduled clinic visit post challenge, their nominated contacts, next of kin and neighbours may be informed and a search started.
- Participants will be counselled to contact the study team or doctor if they feel feverish or unwell in the 6 months following the challenge.

10.7.7. Measures to be taken if a participant goes missing post challenge

In the unlikely event that a participant should (a) fail to attend for a scheduled clinical visit or (b) be un-contactable by telephone after being inoculated with PfSPZ Challenge and before completion of an appropriate course of anti-malaria therapy, the following groups of people will be informed:

- All investigators
- The participant's nominated contact and next of kin
- The trial sponsor
- The local ethics committee
- Local Accident and Emergency departments
- The Safety Monitoring Committee (SMC)

All efforts will be made to locate the participants, including assistance by local authorities. While all parties will aim to preserve the participant's confidentiality, if necessary, details of the participant's identity and participation in the study may be passed to the local and national media

in order to help locate the missing individual. Participants will be informed of this during screening and this information will be placed in the consent form.

10.8. Clinical visits

All clinical visits relate to injection days. Injections are labeled as roman numbers (I, II, III, C) and the relative day preceding or subsequent to injection is given a positive number (e.g. the seventh day after second vaccination II+7, one day before CHMI C-1). The procedures at each visit are given below and the table of study procedures as Annex 1 of the protocol.

10.8.1. Screening and immunization phase

Screening

- Informed consent
- Full medical history
- Physical examination
- Check in-/exclusion criteria
- ECG
- Review of vaccinations
- Urine dip stick
- Urine sampling
- Stool sampling
- Blood sampling (25 ml):
    - CBC
    - Biochemistry (AST, LDH, ALT, creatinine)
    - Virology
    - Serum banking
    - Serum for baseline immunology
    - Thick blood smear

Day of first immunization (I)

Before vaccination:

- Review screening laboratory test results
- Review in-/exclusion criteria
- Assignment of study ID
- AE review
- Symptom-directed physical examination, and examination of the vaccination site(s) for any abnormalities
- Vital signs and axillary temperature
- Concomitant medication
- Blood sampling (50 ml)
    - CBC
    - Biochemistry
    - Serum for immunology
    - PBMC for immunology

Vaccine administration

After vaccination:

- Observe for a minimum of 30 minutes
- AE review

- ID card containing participant's study ID number and photo

Day I+1
- Interview
- Vital signs and axillary temperature
- Urine sampling
- AE review

Days I+2, I+4, I+6
- Home visit by field worker
    - Interview
    - Basic AE review

Day I+7
- Interview
- Vital signs and axillary temperature
- AE review
- Blood sampling (15 ml):
    - CBC
    - Biochemistry
    - Serum for immunology
    - PBMC for immunology

Day I+14
- Interview
- Vital signs and axillary temperature
- AE review
- Blood sampling (15 ml):
    - Serum for immunology
    - PBMC for immunology

Day of second immunization (II)

Before vaccination:
- AE review
- Symptom-directed physical examination, and examination of the vaccination site(s) for any abnormalities
- Vital signs and axillary temperature
- Concomitant medication
- Blood sampling (15 ml)
    - CBC
    - Biochemistry
    - Serum for immunology
    - PBMC for immunology

Vaccine administration

After vaccination:
- Observe for a minimum of 30 minutes
- AE review

Day II+1

- Interview
- Vital signs and axillary temperature
- AE review

Days II+2, II+4, II+6

- Home visit by field worker
    - Interview
    - Basic AE review

Day II+7

- Interview
- Vital signs and axillary temperature
- AE review
- Blood sampling (15 ml):
    - CBC
    - Biochemistry
    - Serum for immunology
    - PBMC for immunology

Day II+14

- Interview
- Vital signs and axillary temperature
- AE review
- Blood sampling (9 ml):
    - Serum for immunology
    - PBMC for immunology

Day of third immunization (III)

Before vaccination:

- AE review
- Symptom-directed physical examination, and examination of the vaccination site(s) for any abnormalities
- Vital signs and axilary temperature
- Concomitant medication
- Urine sampling
- Blood sampling (15 ml)
    - CBC
    - Biochemistry
    - Serum for immunology
    - PBMC for immunology

Vaccine administration

After vaccination:

- Observe for a minimum of 30 minutes
- AE review

Day III+1

- Interview
- Vital signs and axillary temperature
- Urine sampling
- AE review

Days III+2, III+4, III+6

- Home visit by field worker
    - Interview
    - Basic AE reveiw

Day III+7

- Interview
- Vital signs and axillary temperature
- AE review
- Blood sampling (15 ml):
    - CBC
    - Biochemistry
    - Serum for immunology
    - PBMC for immunology

Day III+14

- Interview
- Vital signs and axillary temperature
- AE review
- Blood sampling (15 ml):
    - Serum for immunology
    - PBMC for immunology

Day III+28

- Interview
- Review of AEs
- Physical examination
- Vital signs and axillary temperature of participant
- Concomitant medication
- Urine sampling
- Stool sampling
- Urine dip stick
- Blood sampling (25 ml):
    - CBC
    - Biochemistry (AST, LDH, ALT, creatinine)
    - Virology
    - Serum for immunology
    - PBMC for immunology
    - Thick blood smear

10.8.2. Controlled human malaria infection

Controlled human malaria infection can be postponed up to 14 days.

Days C-7 to C-3

- AE review
- Urine sampling

12-hourly dministration of 300 mg clindamycin base p.o.; total number of doses: 10

Day C-1

- Record any complaints, symptom-directed physical examination, and examination of the injection site for any abnormalities.
- Record vital signs and axillary temperature of participant.
- Record baseline data for solicited general symptoms.
- Record concomitant medication.
- Urine sampling
- Blood sampling (50 ml)
    - CBC
    - Biochemistry (AST, LDH, ALT, creatinine)
    - Serum banking
    - Serum for immunology
    - PBMC for immunology
    - Thick blood smear and qPCR

Day C

- Interview
- AE review
- Review of laboratory values
- Physical examination
- Vital signs and axillary temperature of participant

Injection of 3200 PfSPZ Challenge

After inoculation:

- Observe for a minimum of 30 minutes
- AE review

Day C+1

- Interview
- Vital signs and axillary temperature
- Urine sampling
- AE review

Day C+2 to C+5

- Home visit by field worker
    - Interview
    - Basic AE review
    - Urine sampling

Day C+6 to C+34

- Interview
- Vital signs and axillary temperature

- AE review
- Urine sampling on Day 34
- Blood sampling (2 ml):
    - Thick blood smear and qPCR

Day C+35 and/or Day of malaria

- Interview
- Physical examination
- Vital signs and axillary temperature
- AE review
- Urine sampling
- Blood sampling (25 ml):
    - CBC
    - Biochemistry
    - Serum for immunology
    - PBMC for immunology
    - Thick blood smear and qPCR

Antimalarial treatment initiation

10.8.3. Late follow-up

All follow up visits following C35 can be done within a window of ±7 days of the target schedule.

Day C+56

- Interview
- Physical examination
- Vital signs and axillary temperature
- AE review
- Urine sampling
- Stool sampling
- Blood sampling (15 ml):
    - Serum for immunology
    - PBMC for immunology
    - CBC
    - Biochemistry
    - Serum banking
    - Thick blood smear

10.9. Unscheduled visits

Participants will be advised to report to the research center anytime they feel unwell, regardless of the perceived cause. At such unscheduled visits, a history and physical examination, clinical laboratory tests including malaria smear if indicated, documentation of any AEs and any other medically indicated diagnostic or therapeutic procedures will be performed. These will be recorded as observations in the participant's study record and if the physician finds them to be adverse events, the appropriate CRF sections will be completed.

11. Adverse events and reporting procedures

11.1. Definitions

Definitions for the terms adverse event, adverse reaction, and unexpected adverse reaction have previously been agreed to through the ICH-GCP guidelines and by consensus of the more than 30 collaborating centers of the WHO International Drug Monitoring Centre (Uppsala, Sweden). Although those definitions can pertain to situations involving clinical investigations, some minor modifications are necessary, especially to accommodate the pre-approval, development environment.
The following definitions, with input from the WHO Collaborative Centre, have been agreed upon:

11.1.1. Adverse Event (AE)

An AE is any untoward medical occurrence in a clinical investigation participant, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

11.1.2. Adverse Event Following Immunization (AEFI)

An AEFI is any untoward medical occurrence which follows immunization and which does not necessarily have a causal relationship with the usage of the vaccine. For this study the definitions of AE and AEFI are overlapping and the term AEFI is used for AEs, where causality assessment follows a standardized algorithm and occur until first CHMI (C) [74].

11.1.3. Adverse Drug Reaction (ADR)

In the *pre-approval clinical* experience with a new medicinal product or its new usages, particularly as the dose(s) may not be established, all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions.
The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility; i.e. the relationship cannot be ruled out.

11.1.4. Unexpected Adverse Drug Reaction

An adverse drug reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational medicinal product) is considered as an unexpected adverse drug reaction.
When there is a Serious Adverse Event (SAE) that is unexpected and associated with the use of the medication (Suspected Unexpected Serious Adverse Reaction (SUSAR)), and which occurs during or after treatment, an "expedited report" is to be submitted to the regulatory authority.
Medical and scientific judgment should be exercised in deciding whether "expedited reporting" is appropriate in other situations. These may include important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.
The purpose of "expedited reporting" is to make regulators, investigators, and other appropriate people aware of new, important information about serious reactions. Therefore, such reporting will generally involve events previously unobserved or undocumented. Events will be defined as

"expected" or "unexpected" depending on whether they have been previously observed, rather than if they might be anticipated from the pharmacological properties of a medicinal product.

11.1.5. Serious Adverse Event

During clinical investigations, adverse events may occur which, if suspected to be medicinal product-related (adverse drug reactions), might be significant enough to lead to important changes in the way the medicinal product is developed (e.g., change in dose, population, required monitoring, consent forms). This is particularly true for reactions that, in their most severe forms, threaten life or function. Such reactions should be reported promptly to regulators. Therefore, special medical or administrative criteria are needed to define reactions that, either due to their nature ("serious") or due to the significant, unexpected information they provide, justify expedited reporting. 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 refers to patient/event outcomes 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.

A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

- Results in death;
- Is life threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect.

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

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

All SAEs and the actions taken to treat them must be documented and reported to the sponsor. Even when they are unrelated to the intervention and reporting to the regulatory authority is not required (e.g. an accident that results in hospitalization). Classification of a SAE as SUSAR is done by the sponsor and not by the investigators.

11.1.6. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events and serious adverse events

Abnormal laboratory findings (e.g., clinical chemistry, hematology and urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be staged and recorded as AEs if they meet the definition of an AE. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study, will be reported as AE.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

11.2. Safety monitoring

11.2.1. Role of the local safety monitor:

The Local Safety Monitor (LSM) will be an experienced clinician qualified to evaluate safety data from clinical trials of vaccines. He/she will not be directly involved in the clinical trial and will be based in Lambaréné. All SAEs will be reported to him/her. In exceptional circumstances, for example a death possibly related to vaccination, or under conditions as outlined in below in the safety-monitoring plan, the LSM will have the authority to suspend vaccination pending discussion with the sponsor and collaborators. The LSM will also pass on reports to the Safety Monitoring Committee (SMC) regularly, during the progression of the trial. Thus the LSM's role will include:

- Acting as the study participants' advocate.
- Promptly communicating relevant safety information to the SMC.
- Providing advice to the Investigators on whether a set of clinical circumstances in a study warrants formal notification to the sponsor and SMC.
- Providing clinical advice on any illness in study participants especially in circumstances in which treatment might influence the course of the trial.
- Review all SAEs.

11.2.2. The Safety Monitoring Committee (SMC)

A Safety Monitoring Committee (SMC) will be appointed to provide real-time safety oversight. The SMC will review all SAEs. The SMC will be notified within 24 hours of the sponsor being aware of their occurrence. The SMC has the power to recommend termination of the study to the Sponsor, if deemed necessary following a study intervention-related SAE. Dose escalation of GMZ2-CAF01 from 30 to 100 µg depends on a positive safety review and approval by the SMC as outlined in the SMC charter. Investigator and sponsor will prepare a report with interim data up to Day I14 of the group receiving 30 µg GMZ2-CAF01. In addition to the Chairman of the SMC, there will be a minimum of two other appropriately qualified committee members. The sponsor convenes the SMC *ad hoc* (except for the meeting to transit from 30 to 100 µg GMZ2-CAF01) and all communications with the SMC shall be shared between the sponsor and the PI. The local safety monitor will be one member of the SMC.

11.2.3. Holding Rules

After each vaccination, the data manager will provide tables with safety data to the SMC. The safety tables will be reviewed by the SMC in a blinded manner. If there are no safety issues noted, the study will proceed as planned. However, if based on the review of the safety data tables provided, 50% of participants in a particular group are found to have developed a Grade 3 adverse event, related to vaccination and persisting at Grade 3 for >48 hours during the 14 follow-up days after vaccination, subsequent vaccination of that group will be put on hold pending discussion with the PI and the sponsor.
Within five working days of the Local Safety Monitor placing vaccination of a group on hold, the sponsor will organize a meeting (via teleconference or face-to-face) to review and discuss the safety data and the events leading to the hold order. At least two working days prior to this meeting, the Sponsor will disseminate copies of all relevant safety data to all meeting participants.

Activation of the Holding Rules requires a thorough review by the SMC of blinded reactogenicity and safety data and discussion with the investigator and the sponsor.

11.2.4. Process for restarting vaccination

The vaccination of a group may be put on hold. Continued vaccination of that group may restart only if the sponsor expressly gives authorization to the PI to resume vaccinations.

11.2.5. Process for stopping vaccination of a group or of the trial

In the event that vaccination of a particular group is stopped, the sponsor will inform the EC through the investigator. A report will be written detailing the rationale used for reaching this decision.

11.3. Safety Data Collection and Management Procedures

11.3.1. Expected Adverse Vaccine Reactions

As for any adjuvanted vaccine, local reactions are expected. Systemic reactions are less often observed; however a standardized data collection of adverse reactions will be done.
So far, clinical experience with aluminum hydroxide and CAF01 has shown that the most frequent local reactions are pain, induration, erythema and swelling at the site of injection. Those reactions are usually mild and transient. There is a lack of information concerning expected systemic reactions; therefore a special attention will be given to general signs i.e. fever, irritability or fussiness, drowsiness, and loss of appetite.
In case of a severe local skin reaction (uni- or contra-lateral), a skin biopsy may be performed as part of the clinical evaluation.

11.3.2. Safety Data Collection

All the AEs, whether observed by a clinical investigator or by the participant, must be carefully and accurately documented in the CRF. 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 vaccine.
6. Action taken, including treatment.
7. Outcome.

Safety assessments will be obtained and recorded by the Investigator. "Solicited" and "unsolicited" AEs will be actively followed for 30 minutes after each injection, and at regular intervals during follow-up. Table 2 of Annex lists AEs that will be solicited.

11.3.3. Time period, frequency, and method of detecting adverse events and serious adverse events

All AEs occurring during the study will be recorded; irrespective of severity or whether or not they are considered vaccination-related.
Additionally, SAEs that are related to study participation (e.g. procedures, invasive tests, a change from existing therapy) or are related to a concurrent medication will be collected and recorded from the time the participant consents to participate in the study until the end of the follow-up.
The investigator will enquire about the occurrence of AEs at every during the study and throughout the follow-up phase.
All AEs observed by the clinical team, or reported by the participant spontaneously, or in response to a direct question, will be evaluated. The nature of each event, date and time (where appropriate) of onset, outcome, intensity and relationship to vaccination should be established. Details of any corrective treatment will be recorded on the appropriate page of the CRF.

As a consistent method of soliciting AEs, the participant will be asked non-leading questions such as:

"Have you felt different in any way since receiving the vaccine or since the previous visit?"

AEs already documented in the CRF, i.e. at a previous assessment, and designated as 'not recovered/not resolved' or 'recovering/resolving' should be reviewed at subsequent visits, as necessary. If these have resolved, the documentation in the CRF should be completed.

Note: If an AE changes in frequency or intensity during the specified reporting period, a new record of the event will be entered.

When an AE/SAE occurs, it is the responsibility of the PI to review all documentation (e.g., hospital progress notes, laboratory and diagnostics reports) relative to the event. An investigator will then record all relevant information regarding an AE/SAE on an AE report form. It is not acceptable to send photocopies of the participant's medical records to the sponsor instead of the appropriate completed AE forms. However, there may be instances where the sponsor requests copies of medical records. In such instances, all medical records will be de-identified prior to submission to the sponsor.
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.

11.3.4. Assessment of causality

The clinical investigators are obliged to assess the relationship between investigational product and the occurrence of each AE/SAE. The investigator 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 investigational product will be considered and investigated. The Investigator will also consult the Investigator Brochure and/or Product Information for marketed products, in the determination of his/her assessment.
There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report. It is regardless very important that the PI always makes an assessment of causality for every event prior to transmission of the SAE report form to the sponsor. The PI may change his/her opinion of causality in light of follow-up information, amending the SAE report form accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.
All solicited local (injection site) reactions will be considered causally related to vaccination. Causality of all other AEs should be assessed using the following question:

"*Is there a reasonable possibility that the AE may have been caused by the investigational product?*

NO, the AE is not causally related to administration of the study vaccine. There are other, more likely causes, and administration of the study vaccine(s) is not suspected to have contributed to the AE.
YES, there is a possible or probable cause that the vaccine(s) contributed to the AE. If an AE is "serious" (see definition of serious adverse event), it will be examined to the extent to be able to determine ALL contributing factors applicable to each SAE.
Other possible contributors include:

- Medical history.
- Other medication.
- Protocol required procedure.

- Other procedure not required by the protocol.
- Lack of efficacy of the vaccine(s), if applicable.
- Erroneous administration.
- Other cause (specify).

11.3.5. Medically attended visits

For each solicited and unsolicited symptom the participant experiences, the participant will be asked if they received medical attention defined as hospitalization, an emergency room visit or a visit to or from medical personnel (medical doctor) for any reason and this information will be recorded in the CRF.

11.3.6. Follow-up of adverse events and serious adverse events and assessment of outcome

After the initial AE report, the investigator is required to proactively follow each participant and provide further information to the sponsor on the participant's condition. All AEs and SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be specifically reviewed at subsequent visits/contacts.

Investigators will follow-up participants with SAEs or participants withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, the event is otherwise explained, or the participant is lost to follow-up; or, in the case of other non-serious AEs, until they complete the study or they are lost to follow-up.

Clinically significant laboratory abnormalities will be followed up until they have returned to normal, or a satisfactory explanation has been provided. Additional information (including but not limited to laboratory results) relative to the subsequent course of such an abnormality noted for any participant must be present.

The sponsor may request that the investigator performs or supplemental measurements to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a participant dies during participation in the study, the sponsor will be provided with a copy of any available post-mortem findings, including histopathology.

New or updated information will be recorded on the originally completed SAE report form, with all changes signed and dated by the investigator. The updated SAE report form should be resent to the sponsor within 48 hours of receipt of the follow-up information.

Outcome of any non-serious AE occurring within 30 days post-vaccination or any SAE reported during the entire study will be assessed and classified as:

1. Recovered/resolved.
2. Not recovered/not resolved.
3. Recovering/resolving.
4. Recovered with sequelae/resolved with sequelae.
5. Fatal (SAEs only).

11.3.7. Reporting of Serious Adverse Events

6.4.1.1 Timeframes for reporting of SAEs

SAEs will be reported promptly once the investigator determines that the event meets the protocol definition of a SAE. The Investigator or designate will report to the sponsor within **24 hours** of becoming aware of the SAE. Additional or follow-up information relating to the initial SAE report is also to be reported to the sponsor within 24 hours of receipt of such information. The reporting should be principally by phone; then the investigator should immediately send the completed SAE report to the sponsor alternatively by email or fax, with an acknowledgment of receipt.

6.4.1.2 Completion and transmission of SAE reports

Once an investigator becomes aware that an SAE has occurred in a study participant, s/he will report the information to the sponsor within 24 hours. The SAE report form must always be completed as thoroughly as possible with all available details of the event and signed by the PI (or designee). If the investigator does not have all information regarding an SAE, h/she will not wait to receive additional information before notifying the sponsor of the event and completing the form. The form will be updated when additional information is received and forwarded within **48 hours**.

The investigator must always provide an assessment of causality at the time of the initial report as earlier described.

Initial notification via the telephone does not replace the need for the investigator to complete and sign the SAE Report Form within 24 hours.

In the event of a death determined by the investigator to be related to vaccination, sending of the fax must be accompanied by telephone call to the Study Contact for Reporting SAEs

11.3.8. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to the sponsor in accordance with the procedures detailed above. The sponsor has the responsibility to promptly notify as appropriate, both the regulatory authorities about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the sponsor is essential so that legal obligations and ethical responsibilities towards the safety of other participants are met.

The sponsor, together with the PI will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the EC.

11.3.9. Post study adverse events and serious adverse events

A post-study AE/SAE is defined as any event that occurs outside of the study period. Investigators are not obligated to actively seek AEs or SAEs in former study participants.

If however the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and h/she considers the event reasonably related to the investigational product, the investigator will notify the sponsor.

11.3.10. Treatment of adverse events

Treatment of any adverse event is at the sole discretion of the clinical investigator and according to current good medical practice. Any medication administered for the treatment of an AE should be recorded in the participant's CRF.

The clinical research facility includes private consultation rooms, a procedure room, a resuscitation suite with oxygen, suction and resuscitation kits, and a post-vaccination observation room. An ambulance with suction and oxygen will be immediately available on vaccination days, and may be accessible with a short delay on other days. The pharmacy at CERMEL has sufficient stocks to provide participants with oral and parenteral drugs for the treatment of common illnesses (including malaria and systemic bacterial infections) free of charge, using essential medicines and treatment regimens that meet or exceed standards recommended by the Gabon Ministry of Health. A blood transfusion facility is available on site. Twenty-four hour hospitalization and basic emergency surgery services are available on the CERMEL campus at the Albert Schweitzer Hospital. Twenty-four hour nursing staffing and 24-hour on call physicians will be available.

If the PI or the LSM judges that a participant requires hospitalization, this will be immediately facilitated and the clinical team will ensure that the best possible treatment is offered.

12. Investigational products

12.1. Study Product Description

12.1.1. Description

GMZ2 is a recombinant vaccine. The fusion protein consists of conserved fragments of *P. falciparum* MSP3 and GLURP expressed in *L. lactis*. GMZ2 is produced, purified, vialed and lyophilized according to cGMP guidelines. The vaccine is presented in single-dose vials. The aspect is of a white amorphous powder. A detailed description of the product is provided as part of the IB.

12.1.2. GMZ2 vial content

Active ingredient: GMZ2: 120 µg per vial
Other ingredients: 10 mM KH2PO4 + 137 mM NaCl + 0.6% sucrose, pH 6.5

12.1.3. Manufacturer

GMZ2 vaccine was manufactured at Henogen S.A in Belgium. Lyophilisation was performed at SynCo Bio partners, The Netherlands.

12.1.4. Preparation

The vaccine will be reconstituted with adjuvant according to a SOP. GMZ2-alumm will be prepared as one single dose of 100 µg, GMZ2-CAF01 will be prepared as formulations containing 30 µg or 100 µg GMZ2. Reconstitution with adjuvant will be performed under a laminar flow hood. The adjuvanted solution will be drawn into disposable vanish point syringes for single use with 25 mm, 25 gauge single-use needles. Volume of injection will be 0.5 mL.

12.1.5. Aluminum hydroxide

Alhydrogel is a crystalline aluminum oxyhydroxide (AlO2H), known mineralogically as Boehmite. The structure consists of corrugated sheets of aluminum octahedra. The concentration used for the candidate vaccine is 0.85 mg alum per dose.

12.1.6. CAF01

CAF01 is a liposome-based adjuvant consisting of the immune stimulating synthetic glycolipid TDB (Trehalose-Dibehenate) incorporated into cationic Di-methyldioctadecylammonium bromide (DDA) liposome. The concentration of DDA/TDB will be 625/125 µg per dose.

12.1.7. Control vaccine

Rabies vaccine (Verorab, Sanofi Pasteur) will be used as control vaccine. The product will be obtained commercially and administered according to the manufacturer's specifications in the same schedule as GMZ2 (three injections in four-week intervals).

12.1.8. Precaution for use

A separate sterile syringe will be used for each individual trial participant to prevent transmission of infectious agents. Before injection, the site of injection will be cleansed with a suitable antiseptic and the entire content of each syringe will be used. To prevent injection into a blood vessel, the plunger will be pushed back. Only if no blood is aspirated injection will be performed. Appropriate equipment will be available in case of immediate allergic reactions.

12.2. Vaccination

Enrolled participants will receive three injections of Rabies vaccine, GMZ2-Alum or GMZ2-CAF01 vaccine by intramuscular route into the deltoid muscle region; left arm at the first and third injection, and right arm for the second injection. Caution will be taken to ensure that the

injection does not go into a blood vessel. The site of injection will be recorded on the CRF. In case that one arm is not accessible, the sides may be switched.

12.3. Prior and Concomitant Therapy

At each study visit/contact, the Investigator will question the participants about any medication that may have been taken, including traditional medicines.
Concomitant medication, including any vaccine other than the study vaccines, and any other medication relevant to the protocol, including any specifically contraindicated or administered during the period starting from one week before each study vaccination and ending four weeks after, will be recorded in the CRF. The following data on concomitant medication will be specified and recorded on CRFs:

- Trade name and generic name
- Total daily dose
- Start and stop dates (and if appropriate, time of day)
- Indication (related AE)

Immunosuppressive or -modulatory treatments are exclusion criteria. If it is necessary to use one of them during the trial period, this will be considered as a deviation from the protocol and the data of the concerned participant treated accordingly. Antipyretics or analgesics will not be allowed as preventive treatment of pain or fever before the vaccination but may be used after AE documentation for symptom relieve.
Other vaccinations not mentioned by the trial protocol will not be allowed during the 30 days before the first dose of vaccine. An exception is the receipt of tetanus vaccines, which may be given 14 days or more before or after vaccination. If a vaccination is necessary during the 30-day time window, this will be considered as a deviation from the protocol, and the data of the concerned participant will not be included in the according to protocol analysis.

12.4. Management of Vaccines – GMZ2

The single-dose vials will be labeled with a standard label, mentioning that the vaccine use is restricted to clinical trial use. The pharmacists on site will be responsible for vaccine accountability.

12.5. Storage and Shipment Conditions

Qualified and trained personnel will be personally responsible for product management. The vaccines will be shipped between -80°C and -20°C to the trial site clinics according to the pre-determined schedule. Temperature will be maintained by the use of special cooling boxes under continuous monitoring by automatic temperature recorders. Vaccines will be used only after receipt of approval from the sponsor.
The study staff in charge of the products receipt will return an acknowledgement of receipt to the shipper and the sponsor. The person in charge of product receipt will check that the cold chain and the chain of custody were maintained during shipment. In case of a problem, h/she will alert the clinical trial coordinators and the sponsor immediately. The acknowledgement of receipt will be dated and signed by the person in charge of product management. One copy will be kept archived on site, the other copies will be returned to the trial sponsor and the IP sponsor. GMZ2 candidate vaccine will be stored at a temperature of -20°C. Reconstituted vaccines will be stored at temperatures ranging from +2°C to +8°C (in a refrigerator). Temperature will be monitored daily and documented on an appropriate form up to the shipment of extra vials to the sponsor or designee. The reconstituted vaccines should be administered within six hours. In case of deep freezing or accidental disruption of the cold chain, vaccines will not be administered and the PI

or the responsible pharmacists will contact the sponsor immediately to receive further instructions.

12.6. Management of unused investigational products

Unused and/or open products will be destroyed upon completion of the trial. A SOP for IP handling and destruction will be provided by the manufacturer.

12.7. PfSPZ Challenge

PfSPZ Challenge is composed of aseptic, cryopreserved *P. falciparum* sporozoites used for CHMI trials, produced by the biotechnology company Sanaria, in Rockville, Maryland, USA [77]. In brief, manufacture includes the production, under traditional environmental conditions, of eggs from a colony of *A. stephensi* mosquitoes housed in a controlled environmental chamber. Initial surface disinfection of the eggs is performed by exposure to chemical agents. The eggs are then further disinfected in a Class II biosafety cabinet (BSC) in the clinical manufacturing facility (CMF), a good manufacturing practice (GMP) clean room, using aseptic techniques. All materials and product are handled using aseptic methods at all times to ensure that contaminating microorganisms are not introduced to and carried through the process. Surface-disinfected eggs are inoculated into sterile, vented flasks containing aseptic growth medium. The eggs hatch and develop into pupae, which are transferred to adult mosquito containers where the adult mosquitoes emerge. These adult mosquitoes, which have been raised under aseptic conditions, are fed *P. falciparum* gametocyte-infected blood in a BSC in a High-Security Insectary within the CMF. The *P. falciparum* gametocyte-infected blood has been produced from cultures of the *P. falciparum* strain NF54 derived from a Working Cell Bank of the well-characterized *P. falciparum* strain NF54. Infected adult mosquitoes are maintained under aseptic conditions until *P. falciparum* sporozoites migrate to the salivary glands. The salivary glands from the *P. falciparum* sporozoite infected mosquitoes are removed by hand dissection under aseptic conditions. Salivary glands are then triturated to release the *P. falciparum* sporozoites. The sporozoites are purified, counted, and, at a specified concentration, cryopreserved. Cryopreservation commences with the addition of cryoprotective additives to the purified sporozoites to produce the PfSPZ Challenge product. PfSPZ Challenge is dispensed into screw-cap vials containing 15,000, 50,000, or 100,000 PfSPZ in a 20 μL aliquot. PfSPZ Challenge is stored in liquid nitrogen vapour phase at -150°C to -196°C.

The diluent for PfSPZ Challenge is composed of phosphate buffered saline (PBS) and human serum albumin (HSA). Vials of PBS and HSA will be shipped to the clinical site, where diluent composed of PBS and HSA is prepared according to a SOP. Sanaria manufactures PBS that in compliance with GMP and according to upstream processing specifications. Every lot of PBS is supplied with a Certificate of Analysis that is reviewed and approved by Sanaria. The PBS is stored at ambient temperature in a controlled room.

HSA (25%), approved for parenteral, intravenous administration to humans is purchased by Sanaria and re-aliquoted to smaller volumes. Every lot of HSA is supplied with a Certificate of Analysis that is reviewed and approved by Sanaria. The HAS is repackaged by Sanaria. HSA vials are stored at ambient temperature in a controlled room.

PfSPZ Challenge is stored in liquid nitrogen vapour phase at -150°C to -196°C until it is shipped to a clinical study site. Shipment is in compliance with all U.S. Food and Drug Administration (FDA), U.S. Department of Transportation, and United Nations transport guidelines for shipping bio-hazardous materials on dry ice and liquid or vapour phase nitrogen.

Transfer of PfSPZ Challenge from its storage site to the clinical trial site will follow a Sanaria SOP. At the study site, the liquid nitrogen vapour phase container will be monitored. Receipt of the PfSPZ Challenge will be documented on a Tracking Log by study staff.

Each clinical site must confirm that the vials of PfSPZ Challenge have been transported and stored below -150°C. Immediately prior to use, PfSPZ Challenge in cryovials (each of which

contains 15,000, 50,000 or 100,000 PfSPZ), will be thawed individually or in pairs by partial submersion of the vials for 30 seconds in a 37°C ± 1°C water bath. Designated, trained study staff will then prepare, dilute (if necessary) and dispense PfSPZ Challenge to clinical staff at the clinical study site according to a SOP provided by Sanaria. The PfSPZ Challenge containing a particular dose to be administered to each participant will follow study-specific SOPs.
PfSPZ Challenge will be administered by direct venous inoculation (DVI) 0.5 ml through a 25G needle with a 1 ml syringe according to standard operating procedures. An IV catheter will be put in place at a different site to be able to administer fluids and drugs in case of severe adverse reactions. The study staff administering PfSPZ Challenge will wear gloves and appropriate protective clothing. During administration of PfSPZ Challenge, Advanced Life Support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis.
Material used to prepare and administer PfSPZ by DVI will be disposed according to relevant guidelines. A dressing will cover the IV injection site at least until the injection site stops bleeding.

13. Evaluation criteria

13.1. Primary evaluation criterion: Safety

Safety will be evaluated using standard toxicity scales for adults [78]. Grading lists are given as Table 2 of the Annex.
Laboratory abnormalities will be assessed for their clinical significance and graded according to an adapted toxicity scale. All relevant laboratory measurements will be done at CERMEL. In case tests are not available, samples are shipped to Tübingen and measured at the central laboratory of the university clinics.

13.2. Secondary evaluation criteria: Efficacy

Protective efficacy will be assessed by CHMI with PfSPZ Challenge, strain NF54. Inoculation and follow up is done according to guidelines and established procedures with daily thick blood smears to calculate time to first parasitemia and time to malaria. The follow up will be censored on Day 35 after PfSPZ Challenge injection.

13.3. Secondary evaluation criteria: Immunogenicity

Immunological assays will be performed according to a separate immunological manual and SOPs. Serum and plasma will be stored on site at the appropriate temperature (-20 and -80°C) and storage will be monitored. Cryopreserved PBMC will be stored at -150°C or liquid nitrogen. Transport of samples will be done according to local, national and international guidelines.

14. Statistical Considerations

Safety and tolerability data is presented as descriptive analysis, as listing and graphically. No formal hypothesis testing will be done. The level of significance is set at a two-tailed type I error alpha <5%. Statistical analyses will be detailed in a separate statistical analysis plan (SAP). Sponsor and PI must approve the SAP before the study is unblinded.

14.1. Sample size justification

The total number of participants will be 50. These will be split into four groups with:

- 8 healthy adults receiving 30 µg of the GMZ2-CAF01.
- 22 healthy adults receiving 100 µg of the GMZ2-CAF01.
- 12 healthy adults receiving 100 µg of the GMZ2-ALUM.
- 8 healthy adults receiving the control vaccine.

The sample allows detection of safety and tolerability problems in the 100 µg GMZ2-CAF01 groups that occur very frequently (approximately 13%).

To detect a difference of 1.5 times the standard deviation of vaccine-induced antibodies between the 100 µg GMZ2-alum and GMZ2-CAF01 with a power of 90% and a two-tailed alpha of 5% using a t-test, 11 participants per group are required. This would allow one participant to be excluded (e.g. because incomplete vaccination).

To test efficacy the number of participants who develop malaria will be compared. Under the same conditions as for immunological measurements (power: 90%, alpha: 5%), 12 vaccinees and 8 control vaccine-recipients are required to detect a change from 25% to 92% protection against malaria. In addition, time to parasitemia and time to malaria will be compared using Kaplan Meier statistics and a Cox proportional hazards model.

The testing procedure will be hierarchical. Hence no correction for multiple testing will be required.

14.2. Populations

14.2.1. Definitions

According to protocol population

The according to protocol population (ATP) consists of participants who received all vaccine injections within the appropriate schedule and completed clinical visits in time.

All participants who have not been compliant with the protocol will be excluded from this analysis but will be described in the final report. The protocol compliance criteria are the following:

- Participants fulfilling the inclusion and exclusion criteria.
- Participants who have received the correct number of vaccination at the correct time-intervals, as described in the protocol.

Intention to treat population

All participants who have received at least one vaccine dose will be included in the analysis. However, if a participant is not vaccinated for reasons unrelated to the primary outcome measure and the participant's code was broken during the period of the study, that participant will be excluded from further analyses.

Safety population

All participants who received at least one injection of GMZ2 or control vaccine.

14.2.2. Population used for analysis

The primary analysis will be conducted on all participants who have received at least one vaccine dose (ITT).

Analysis of the secondary and exploratory outcome measures will be performed on the ATP population.

14.3. Data Management

The PI or his designee will be the data manager with responsibility for delegating the receiving, entering, cleaning, querying, analyzing and storing all data that accrues from the study. All data will be entered in paper case record forms and transcribed into a validated electronic data capture system that is designed for clinical trials.

All files and source documents will be kept confidentially in locked safety cabinets. Electronic data capture is done with a dedicated database system that fulfills regulatory requirements and follows Clinical Data Interchange Standards Consortium (CDISC) guidelines. The PI and co-

investigators will have access to records. The investigators will permit authorized representatives of the sponsor, regulatory agencies and the monitor to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. At the end of the study, all safety, laboratory data and efficacy data in the clinical database, as well as an audit trail will be transferred to the sponsor.
The clinical trial monitor will check and verify 100% of data on CRFs with source documents, and all identified errors will be corrected if information can be retrieved. After integration of all corrections in the complete set of data, the database will be locked and saved before being released for statistical analysis. An audit trail is maintained throughout the study and data entry will be tracked to the individual entry clerk level. Database integrity will be checked regularly and back ups are maintained at two different locations.

15. Ethics/protection of human participants

15.1. Ethical standard

The Comité National d'Ethique de la Recherche (CNER) is a legally mandated entity by the Gabon government to review and approve research involving human subjects. Regulatory approval will be requested from the Direction Générale de la Santé, Ministère de la Santé, Gabon. Before submission to local and national authorities and committees, the protocol will be reviewed by the Scientific Committee of CERMEL.
Documentation of approval by the ethical, regulatory and scientific review boards will be kept in the investigator's and sponsor's files. Moreover, the study vaccines will only be shipped to the site after the sponsor acknowledges receipt of ethical and regulatory approval. This trial will uphold the standards as articulated in the major ICH-GCP and ethical guidelines. To ensure ethical conduct of the trial, all the clinical investigators will be required to have a certificate of research ethics training at a minimum.
The investigators will inform EC and sponsor of the following:

- All subsequent protocol amendments, informed consent changes or revisions of other documents originally submitted for review.
- Serious and/or unexpected adverse events occurring during the study, where required.
- New information that may affect adversely the safety of the participants or the conduct of the study.
- An annual update and/or request for re-approval, where required.
- When the study has been completed.

15.2. Informed Consent Process

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specified procedures or interventions are carried out.
Information will be given in both oral and written form whenever possible. Independent witnesses will be required to attest that illiterate potential participants have understood the contents of the informed consent.

15.3. Screening and study informed consent

Informed consent is considered to be a dynamic, ongoing process, with continuous availability of investigators to answer any questions that arise in the course of the trial and to ensure that participants understand trial procedures. Should new data become available that could affect participant safety and/or willingness to continue in the study, informed consent would be obtained and documented again.
The extensive contact between the team of investigators and the population of Lambaréné has led to the development of mutual trust.

A period of approximately three weeks is allotted for screening and recruitment to allow enough time for participants to consider their decision about participation and to discuss their participation with family members and others in the community. At the times of screening and recruitment, the form will be given to participants who speak French, and translated orally into the local dialect of each participant who is illiterate. All relevant information on the participant responsibilities including the possible risks such as discomfort and pain at injections and the fact that an experimental vaccine will be tested is explained in detail. In all cases, the investigator will give the participants ample opportunity to inquire about the details of the study and to ask any questions before dating and signing the consent forms, including the opportunity to take a copy of the consent form home to review with family members or others before returning on a later day with their decision. All illiterate participants will have the study and consent forms explained to them point by point by the interviewer in the presence of a witness who will sign the consent form. The witnesses will have no association with the conduct of the study and will not be related to the study participant; efforts will be made to secure confidentiality for participants.
Informed consent will be documented by the use of a written form approved by the EC and reviewed by the SRC, signed or thumb-printed and dated by the participant and by the person who conducted the informed consent discussion. Thumb printing will be used for illiterate persons only, who are expected to constitute the majority of participants. The signature/thumbprint confirms that the consent is based on information that has been understood. Each signed informed consent form will be kept on file by the investigator for checking by the clinical monitor and possible inspection by regulatory authorities. The participant will receive a copy of the signed and dated written informed consent form and any other written information provided by the Investigator, and will receive copies of any signed and dated consent form updates and any amendments to the written information.

15.4. Participant Confidentiality

Participant confidentiality will be strictly upheld by the participating investigators, their staff, and the sponsors and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Before study start, all members of staff on the investigator team will sign an agreement to this effect.
The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsors.
The Study Monitor or other authorized representatives of the sponsors may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

15.5. Future Use of Stored Specimens

The residual sera and cells remaining after the serological and cellular assays described in this protocol, may be used for additional immunological and in vitro studies related to malaria. Permission will be expressly granted for preserving samples for future studies at the time of informed consent at study enrolment. To ensure appropriate storage, the samples may be shipped to Tübingen and other collaborating centers. A material transfer agreement will be proposed by the CERMEL.

15.6. Publication Policy

This protocol will be registered with the US ClinicalTrials.gov registry and the relevant registration number NCT updated once obtained.
The PI, in collaboration with the trial coordinators prepares the final report. It will be signed by the PI and the sponsor representative. The protocol and data derived from the trial are the exclusive property of the sponsor. Any publication or presentation related to the trial must be

approved by the sponsor before submission of the manuscript. The PI is expected to lead the process of peer reviewed journal publication, and this shall be done within six months of completing the clinical study report.

16. Quality assurance and quality control

16.1. QA/QC Policy

CERMEL's current SOPs for quality management, will be reviewed and updated, and used to train appropriate personnel and kept on file with documentation of the training. Data will be evaluated for compliance with protocol and accuracy in relation to source documents. The trial will be conducted in accordance with procedures identified in the protocol. The types of materials to be reviewed, who are responsible, and the schedule for reviews will be referenced in the SOPs. Study-specific training will be provided for all staff prior to the commencement of the trial.

Regular monitoring will be performed according to ICH-GCP. Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, ICH-GCP, and the applicable regulatory requirements. Reports will be submitted to the sponsor about monitoring activities and specific recommendations will be implemented to improve the quality of the trial. The investigational sites will provide direct access to all trial related source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities as the case may be.

16.2. Modification/Amendment of the Protocol

No substantial amendments to this protocol will be made without consultation with, and agreement of the sponsor. Any changes that appear necessary during the course of the trial must be discussed by the investigators and sponsor concurrently. If agreement is reached concerning the need for an amendment, it will be produced in writing by the sponsor and/or the investigators and will be made a formal part of the protocol. An amendment may require EC approval. All amendments must also be transmitted to the regulatory authorities, if applicable. An administrative change to the protocol is one that modifies administrative and logistical aspects of a protocol but does not affect the participants' safety, the objectives of the trial or its progress. An administrative change does not require EC approval. However, the EC must be notified whenever a significant administrative change is made. Approval must be sought for all amendments involving a change in the study procedure. The PI is responsible for ensuring that changes to an approved trial, during the period for which EC approval has already been given, is not initiated without EC review and written approval except to eliminate apparent immediate hazards to the participant.

16.3. Archiving

The trial site must keep all trial documents for at least 10 years after the completion or discontinuation of the trial. The PI will inform the sponsor should there be need for any changes. In the event of lack of storage capacity, the PI shall report to the sponsor to make specific archival arrangement according to sponsor SOPs and applicable regulatory requirements.

16.4. Access to documents

SOPs will be used at all clinical and laboratory procedures. Regular monitoring will be performed according to GCP-ICH (e.g., procedures, data and ethics compliance). The monitor will

undertake 100% source document verification on pre-defined data. Direct access to all trial related documents (e.g. source data, SOPs, case files CRFs) will be ensured for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

16.5. Study personnel

Only appropriate persons qualified by training and experience will be responsible for study procedures. The study personnel file will maintain up to date CVs and a training log of all staff, which will be in the Investigator file as part of the essential documents. Only authorized staff will be allowed to write on trial documents. An authorized signatory list will also be maintained in the investigator file.

16.6. Investigational procedures

The investigators will maintain detailed SOPs for vaccine management, data management, laboratory and clinical procedures. All staff and investigators will be trained in the SOPs relevant to their duties. Copies of SOPs will be available for inspection, audit and review by the sponsor and study monitors. During the study SOPs may be modified to improve them and new SOPs may be developed as needed to improve operations and ensure adherence with the protocol. Some SOPs have been standardized for all sites, for example those for microscopy (which is used to determine the primary end point), while other procedures will be site specific.

16.7. Monitoring

Monitoring visits will be conducted before the study initiation visit and routinely.

16.7.1. Before the study initiation visit

This monitoring visit will ensure that the site is ready to undertake the trial. The clinical monitor will ensure that all designated staff understands relevant SOPs and all requirements are in place. A trial specific training and orientation to GCP will be undertaken.

16.7.2. Routine monitoring visit

A routine monitoring visit will be performed after the first, second and third vaccination as well as at day 140 of the study. The monitor's duty will include, but is not limited to the following:

- Carry out a quality control assessment of trial progress: Adherence to protocol and operating guidelines, data collection, signature of consent forms, completion of documentation, any SAEs, sample and product management, cold chain monitoring.
- Collect the CRFs and correspondent correction sheets.
- Assess the inclusions in order to evaluate the number of complete or on-going observations.

The monitor will check that the trial is progressing according to protocol and ICH-GCP. Any deviations will be reported and appropriate solutions proposed for any problems observed.

16.8. Study close out visit

A close-out visit will be performed for each site at the end of the trial. The objectives of this visit will be to ensure that:

- The centre has all the documents necessary for archiving.
- All samples have been shipped.
- All unused material has been recovered.
- All products have been returned to the sponsor.

17. Financing and insurance

17.1. Financing

The study will be funded partly by support from the German Ministry of Education and Research (BMBF).

17.2. Insurance

The trial will be covered by a clinical trials insurance.

17.3. Compensation of participants

The participants will be granted compensation for losses due to participation in this trial, e.g. lost income due to missing a day's work, transportation to scheduled visits etc. The compensation will be defined based on the population habitude and the number of visits. Short visits will be compensated with 7,500 francs CFA, long visits with 25,000 francs CFA. The total amount of compensation is up to 450,000 francs CFA in case that a participant attends all clinical visits.
The clinical trial insurance policy will be the medium through which appropriate compensation would be accorded in case of trial related events or disability. Any participant experiencing an event related to the trial that causes such disability will be eligible to claim compensation.

17.4. Treatment costs

Treatment costs will be covered by project funds. Costs for hospitalization are covered either by project funds or the insurance (depending on the reason for hospital admission).

18. Termination of the trial

The trial sponsor has the right to terminate the trial. The trial may be discontinued if new data about the investigational product resulting from this or any other trials become available which dictates that the product is not safe for human use. It may also be discontinued if the sponsor so advises based on administrative reasons or other advice from the SMC, or when the regulatory authority so decides. If a trial is to be prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the regulatory authorities and the EC of the reason for termination.
Should the PI deem termination inevitable, he may suspend trial activities while immediately resolving issues arisen with the sponsor.

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Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials
[http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/vaccines/ucm074775.htm]

20. Annex

Table 1: Study procedures

	Screening	I	I1	I2 I4 I6	I7	I14	II	II1	II2 II4 II6	II7	II14	III	III1	III2 III4 III6	III7	III14	III28	C-7 to C-3	C-1	C	C1	C2 to C5	C6 to C34	C35 or M	C56
Informed consent	X																								
Full medical history	X																								
Physical examination	X	X					X					X					X		X	X				X	X
In-/exclusion criteria	X	X																							
Review of vaccinations	X																								
Assignment of ID		X																							
Vaccine injection		X					X					X													
PfSPZ injection																									
Clindamycin (2x300 mg)																		X							
Randomization		X																							
Supply of ID card		X																							
Virology[1]	X																X								
AE review		X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X	X		X	X	
Concomitant medication		X															X		X	X					
Home visit[2]				X					X					X								X			
Sampling (approx. ml)	25	50			15	15	15			15	15	15			15	15	25		50				2	25	15
Complete blood count	X	X			X		X			X		X			X		X		X					X	X
Biochemistry[3]	X	X			X		X			X		X			X		X		X					X	X
Serum banking	X																		X						X
Thick blood smear	X																X		X				X	X	X
Serum for immunology	X	X			X	X	X			X	X	X			X	X	X	X	X					X	X
PBMC		X			X	X	X			X	X	X			X	X	X	X	X					X	X
Urine test	X																X								X
Urine and stool sample	X	X	X#				X	X					X#				X	X*	X		X	X**	X#*		X

Safety relevant procedures can be made out of schedule if a member of the study team or the local safety monitor decides; 1: HIV, Hepatitis B, Hepatitis C; 2: Includes a basic AE review; 3: AST, ALT, creatinine; M: malaria; the total amount of sampled blood will not exceed 500 ml. * Only at day C-3, **Only at Day C5, # urine only, #* Only on day 34 or day of malaria

Table 2: Table of solicited systemic and local Adverse Events

Adverse Event	**Intensity grade**	**Parameter**
Pain at injection site	0	Absent
	1	Minor reaction to touch
	2	Moderate reaction to touch
	3	spontaneously painful
Swelling at injection site*		Record greatest surface diameter in mm
Induration at injection site*		Record greatest surface diameter in mm
Erythema at injection site*		Record greatest surface diameter in mm
Contra-lateral reaction*		Record greatest surface diameter in mm
Pruritus at injection site	0	Absent
	1	Easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
	2	Sufficiently discomforting to interfere with normal everyday activities.
	3	Prevents normal, everyday activities.
Fever	0	axillary temperature < 38°C
	1	38≤ and <38.5 °C
	2	38.5≤ and <39 °C
	3	≥ 39 °C
Irritability/Fussiness	0	Behavior as usual
	1	no effect on normal activity
	2	interferes with normal activity
	3	prevents normal activity
Drowsiness	0	Behavior as usual
	1	Drowsiness easily tolerated
	2	Drowsiness that interferes with normal activity
	3	Drowsiness that prevents normal activity
Loss of appetite	0	Appetite as usual
	1	Eating less than usual/ no effect on normal activity
	2	Eating less than usual/ interferes with normal activity
	3	Not eating at all
Diarrhea	0	None
	1	With no dehydration
	2	With some dehydration
	3	With severe dehydration

Table 3: Summary of time periods between which different classes of concomitant medication/treatment/vaccination must be recorded

Time in relation to vaccination	**Treatments to be recorded**
3 months prior to Dose 1 → Dose 1	All treatments listed as elimination criteria
Screening → 30 Days post Dose 3	All antipyretic, analgesic, antibiotic and any treatments listed as elimination criteria
31 Days post Dose 3 → Final Study Visit	All treatments listed as elimination criteria
One week prior each dose → 30 days post each dose	All medication