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Controlled human malaria infection to evaluate the efficacy of the asexual blood-stage malaria vaccine candidate GMZ2-CAF01

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Do the best you can until you know better. Then when you know better, do better.

– Dr. Maya Angelou

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List of Acronyms

ACT Artemisinin-based Combination Therapy

ADCI antibody-dependent cellular inhibition

AE adverse event

AEFI AE following immunization

BMBF German Ministry of Education and Research

BMI body mass index

CAF01 Cationic Adjuvant Formulation 01

CERMEL Centre de Recherches Médicales de Lambaréné

CHMI Controlled Human Malaria Infection

CSP Circumsporozoite Protein

DVI direct venous inoculation

DZIF German Center for Infection Research

ECG Electrocardiogram

EMA European Medicines Agency

G6PD Glucose-6-Phosphate Dehydrogenase

GCP Good Clinical Practice

GLURP Glutamate Rich Protein

GMZ2 Recombinant *Lactococcus lactis* Hybrid GLURP and MSP3

HPF high power fields

ICH The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use

MedDRA Medical Dictionary for Regulatory Activities

MSP3 Merozoite Surface Protein 3

NAI naturally acquired immunity

PCR Polymerase Chain Reaction

PfEMP1 *P. falciparum* Erythrocyte Membrane Protein 1

PfSPZ *Plasmodium falciparum* sporozoites

qPCR quantitative Real-Time Polymerase Chain Reaction

SAE serious adverse event

sHLA soluble Human Leukocyte Antigen

TBS thick blood smears

VSA variant surface antigens

WHO World Health Organization

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1 Introduction

1.1 Epidemiology of Malaria

Malaria is a life-threatening protozoan parasitic disease transmitted by mosquitoes, which infected approximately 241 million people (95% CI: 218-269 million) worldwide in 2020, of which 627,000 people died [2]. 94% of these fatalities occurred on the African continent [2]. Malaria is one of the most significant causes of infant mortality, depicted by the calculation that it is "taking the life of a child every 2 minutes" [3].

Control efforts in many regions of the world have been able to eradicate or at least reduce the incidence of malaria, with achievements predominantly starting in areas that are further away from the equator and regions with tropical climates lagging behind, with the exception of some islands and very rapidly developing small countries [4]. Recommendations on how to eradicate malaria are numerous, but identification of the foci of infections and elimination of these is emphasized, as well as treating symptomatic as well as asymptomatic cases, and controlling the vectors of the disease [5].

More than $3 billion was spent on malaria control programs, including insecticide-impregnated bed nets, the spraying of insecticides, and diagnosis and treatment of malaria, globally in 2020 [2]. The economic costs of these programs versus the cost of the distribution of an effective vaccine have to be further researched. Fundamentally, primary prevention through the usage of bed nets, the reduction of the infectious mosquito populations, as well as the prevention of the disease through vaccination would be favorable approaches to controlling malaria. One study calculated that a vaccine with 85% efficacy alone could prevent 5.1 million severe malaria cases and 1.1 million deaths within a timeframe of 10 years [6].

Therefore, various studies are being executed in order to develop a safe and effective vaccine.

1.2 Life Cycle of Malaria

Plasmodia are parasitic protozoa, of which five different species cause different forms of malaria in humans: *P. ovale*, *P. vivax*, *P. malariae*, *P. falciparum*, and *P. knowlesi* [7–10]. *P. falciparum* is responsible for 99% of all fatalities and is the species upon which this thesis focuses [2].

Approximately 70 of 462 formally identified *Anopheles* mosquito species are relevant in the transmission of malaria to humans [11], with the *An. gambiae* complex, *An. arabiensis*, and *An. funestus* playing a dominant role [12, 13]. Only the females act as the vector by feeding on human blood and, from their salivary glands through their proboscis, will inject *Plasmodium* sporozoites into the human, who acts as the host for the parasite. After injection the sporozoites travel to the hosts' liver within 30 minutes and enter the hepatocytes, where they multiply asexually to merozoites. One hepatocyte can contain up to 30,000 merozoites [14, 15].

P. vivax and *P. ovale* are thought to be able to remain dormant in the liver as hypnozoites for years [16]. However, in *P. falciparum* infections the hepatocytes usually erupt after 5 - 12 days, which is where the exo- or pre-erythrocytic cycle ends [17]. The free merozoites invade erythrocytes in the bloodstream, where they evolve into trophozoites and then to schizonts, by asexual reproduction. Mature schizonts contain 16 to 32 merozoites, which cause the erythrocytes to burst and release the merozoites into the bloodstream where the process of erythrocyte invasion is repeated [17]. This part of the life cycle is called the erythrocytic cycle and is when the disease becomes apparent to the human, causing typical symptoms

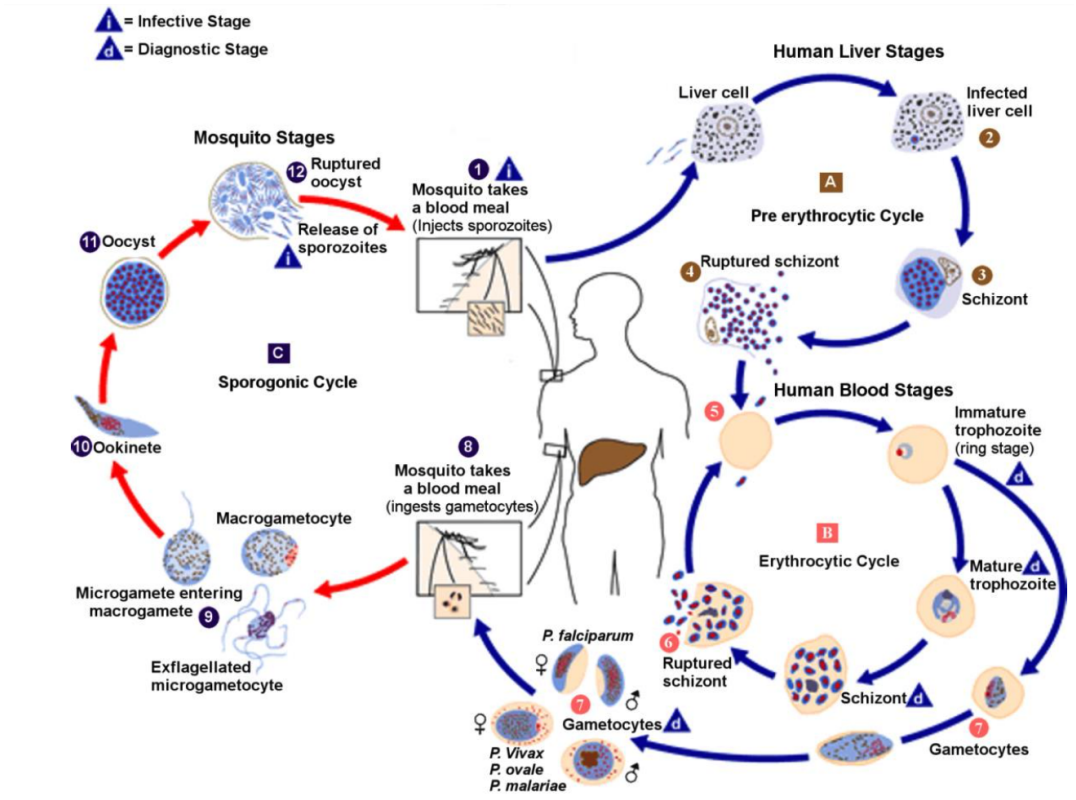

Figure 1: Malaria Life Cycle. Figure from the Centers of Disease Control (CDC) [19]. A mosquito takes up gametocytes through ingestion of blood from an infected human. Inside the mosquito the sporogonic cycle occurs, in which the gametocyte evolves to a sporozoite. Through the next blood meal of the mosquito, the sporozoites are injected into the human and make their way to the human liver, where the pre-erythrocytic cycle occurs. Schizonts evolve within the hepatocytes, they erupt, and the merozoites enter the bloodstream, where they enter erythrocytes to become trophozoites. These again evolve to schizonts which again cause the erythrocytes to erupt. Some merozoites develop into gametocytes which are then again taken up by mosquitoes.

such as fever.

However, some merozoites develop into male and female gametocytes, which are then taken up by mosquitoes. In the gut of the mosquito, they evolve into gametocytes, where a male and female unite to differentiate into zygotes, ookinetes, oocysts, and then to sporozoites, which subsequently migrate to the salivary gland of the vector. Here, the cycle back into the human host can be repeated with the next feeding of the mosquito. Figure 1 depicts the lifecycle of the *Plasmodium* parasite.

The development cycle is dependent on temperatures above 16 °C, which explains why malaria is only endemic in certain regions of the world [18].

1.3 Clinical Presentation of Malaria

A malaria infection becomes apparent to the human host in the asexual erythrocytic phase of the parasite's life cycle. Symptoms commonly emerge after an incubation time of 8 to 15 days (or up to five weeks, if infected by *P. malariae*), starting with unspecific signs, such as chills and high fever. The fever can present in parasite-specific cycles, when the merozoite-releases are synchronized: *P. vivax* and *P. ovale* cause benign malaria, where fevers reoccur every 48 hours; *P. malariae* causes malaria quartana and fevers reoccur every 72 hours; lastly, *P. falciparum* causes the most deadly malaria, falciparum, with unsynchronized fevers. Mixed infections of two or three of the parasites are also possible but tend to be more frequent in patients living in endemic areas.

Apart from chills and high fever, symptoms may include headache, myalgia, arthralgia, tachycardia, fatigue, diarrhea and gastrointestinal dysfunction or abdominal pain, nausea, vomiting, rigor, sweats, jaundice, chest pain, and lower back pain.

Hepatosplenomegaly is common in malaria-endemic regions and is caused by repeated infections. Extent and frequency of hepatosplenomegaly is sometimes described as the malariometric index indicating high transmission intensity in these regions [20]. Malaria is thus a multisystem disorder.

However, the clinical spectrum of symptoms is large and all *Plasmodium* species can cause uncomplicated malaria, defined by the presence of parasites in the patients' blood and absence of signs for severe infection [2]. Complicated malaria is almost exclusively induced by *P. falciparum* and entails a list of indicators defined by the WHO, including but not limited to cerebral malaria, pulmonary edema and acute respiratory distress syndrome, severe anemia, renal failure, hypoglycemia, seizures, and coma [21]. If untreated, malaria tropica can develop into severe malaria and can lead to death within a few days after onset of symptoms. Severe malaria can cause cerebral malaria causing neurological symptoms but also multiorgan failure.

After cerebral malaria, residual symptoms may be hemiparesis, cognitive dysfunction, cerebral ataxia, aphasia, spasticity, and a variety of other neurological symptoms. However, most episodes of severe malaria do not result in major permanent deficits.

These severe progressions of the disease are those commonly reported in the media, as they are the ones causing the health crisis depicted by the high mortality and morbidity described in Section 1.1. However, there are also many asymptomatic and uncomplicated malaria cases that underly various semi-immunity mechanisms, as Section 1.6 will illustrate.

1.4 Diagnosis of Malaria

The diagnosis of malaria can be achieved through light or fluorescence microscopy, rapid diagnostic tests, or nucleic acid amplification techniques.

For the assessment of the necessity of treatment, the World Health Organization (WHO) recommends that all suspected malaria cases be confirmed by either microscopy or, if a trained microscopist is not available, by rapid diagnostic tests [22]. These guidelines are formulated in order to attain the goal of saving human lives without causing excess drug resistances by over-treatment. They are adjusted to real-life settings, which can often be in remote areas with limited resources, where the goal of attaining a result within two hours should be met. However, for scientific research, more advanced techniques can be utilized that are too expensive for routine clinical management, but more accurate, precise, and sensitive to maximize study result validity. The most common methods are briefly described here:

- **Microscopy:**

  This relatively inexpensive method is considered the gold standard and has been used for over 100 years [23]. A thick and thin blood film is produced on a microscope slide. The procedure is done by taking blood samples, either by finger prick or venipuncture [24, 25]. The films are stained with Giemsa stain and then examined under the microscope to identify the presence of parasites (thick blood film) and the parasite species (thin blood film), as well as to count them to make a statement regarding the number of parasites/µL of blood. Detection limits vary between 4-100 parasites/µL of blood [26]. The prerequisite of this method is the presence of a competent microscopist and an appropriate microscope.

Data collection through microscopy is said to underestimate the "prevalence by 50.8 % compared with PCR", with especially high underestimations occurring in regions of low transmission [27].

- **Nucleid Acid Amplification Techniques:**
  Nucleid acid amplification techniques are often not readily available in resource-limited settings, as the equipment is expensive, trained staff must be available, and the reagents to run the machines need to be acquired and properly handled. Polymerase Chain Reaction (PCR) was developed by Kary B. Mullis in 1983 [28]. While PCR is often not used in the clinical setting, it has high value for scientific research due to its high sensitivity and ability to detect up to 0.02 parasites/µL [29].

- **Rapid Diagnostic Test:**
  In comparison to other diagnostic methods, rapid diagnostic tests are the simplest and fastest (5-20 mins) to perform, requiring 5-15µL of blood to be placed on a strip containing an immunochromatographic assay with monoclonal antibodies against target parasite antigens [30]. They require no expensive machinery nor electricity, and limited training to be performed. However, the accuracy of rapid diagnostic tests varies greatly, with false positive results being a common issue.

Thus, as diagnosis can be difficult to be achieved, especially in rural areas, and malaria treatment is also given for fevers unrelated to malaria, causing resistances; a vaccine would be a promising approach to eliminate the disease.

1.5 Treatment of Malaria

Since 2006, the WHO has released a series of *Guidelines for the Treatment of Malaria*, of which the most recent (at the time of this study) (third) edition was published in 2015 [22]. As one of the core principles, it underlines the importance of the use of Artemisinin-based Combination Therapy (ACT) in order to avert the progression of resistance development to currently-available therapies. Furthermore, for uncomplicated malaria, the following ACTs are recommended: artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, dihydroartemisinin plus piperaquine, or artesunate plus sulfadoxine-pyrimethamine [22]. The choice of combinations is based on the individual regions' statistics on drug resistance [22]. For the treatment of severe malaria, the guidelines recommend as first-line treatment the intravenous or intramuscular application of artesunate, followed by an oral course of clindamycin, atovaquone-proguanil or doxycyclin, the oral continuation of the therapy [22]. If artesunate is not available or if it is contraindicated, intravenous quinine is recommended as second line therapy [22]. Due to the better efficacy and better side effect profile, however, artesunate should be the drug of choice if available [31, 32].

1.6 Protective Immunity against Malaria

Since malaria has been a burden to the human population for thousands of years, it is of no surprise that human genetic factors providing protection against malaria have spread in affected populations. Some of the wider-spread resistance mechanisms are haemoglobinopathies, such as the sickle cell mutation and α- and β-thalassaemia, negativity for Duffy blood group expression, or Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency.

Furthermore, there is also naturally acquired immunity (NAI). First noted by western physicians in colonial times, people understood that humans coming from non-endemic malaria regions of the world were at higher risk of acquiring and dying from malaria than indigenous people in holoendemic malaria regions. Later, Robert Koch found that this partial protection only held true for people who were constantly exposed to the parasite. One study estimated that it takes around 10-15 years living in a holoendemic malaria region and being infected around five times per year to acquire such immunity [33]. If individuals who had thus far benefitted from such immunity then left the region of high malaria risk, they would lose this acquired trait. Experiments have been performed in which gamma-globulin from the serum of semi-immune individuals were transfused to malaria-naive individuals. These patients subsequently presented with less severe symptoms and parasitemia, further hinting at this protective effect that local populations in these regions acquire during their life [34–36]. For the development of a vaccine, it is essential to understand the underlying mechanisms that promote this semi-immunity, or NAI, as they try to mimic this process. To date, however, the mechanism of semi-immunity has not been fully understood, which is likely a major factor as to why vaccine development has taken so long to produce satisfactory candidates. The most recent hypotheses to the theory behind the development of semi-immunity are presented in the following.

Different antigens have been identified that are likely to induce the immune response in people who are repeatedly infected with malaria parasites, potentially playing a key role in the acquisition of NAI. Among others, these are the polymorphic blood stage antigens Merozoite Surface Protein 3 (MSP3), erythrocyte-binding antigen 175 (EBA-175), and Glutamate Rich Protein (GLURP) as well as the variant surface antigens (VSA) *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) and *P. falciparum*-encoded repetitive interspersed families of

polypeptides (RIFINs) [37]. Other blood-stage antigens include the apical membrane antigen 1 (AMA1), merozoite surface protein (MSP) 1, MSP2, ring-infected erythrocyte surface antigen (RESA), and serine repeat antigen (SERA5).

As the vaccine candidate discussed in this thesis consists of MSP3 and GLURP, these will be the ones elaborated upon here. In previous studies, MSP3 and GLURP have both been shown to trigger antibody-dependent cellular inhibition (ADCI) [38, 39]. $MSP3_{212\text{-}380}$ is a 48kDa protein expressed on the merozoite surface. A study performed in Burkina Faso was the first to show the immunogenic potential in human beings [40, 41]. $GLURP_{27\text{-}500}$ is a 220kDa protein expressed in both the pre-erythrocytic as well as erythrocytic stages [42].

1.7 Malaria Vaccines

In the past, the world successfully joined forces to eradicate smallpox, closely followed by the fight against poliomyelitis. Other diseases have also been reduced significantly through vaccine programs. What these effective measures all have in common, is that they are directed against viruses or bacteria. No parasite vaccine has been able to produce similar results to date, as parasites have much more complex life cycles, which complicate the identification of a potential vaccine target. Scientists have worked on the development of a malaria vaccine for decades, with dozens of failed attempts lining the way. However, as mentioned in Section 1.6, the phenomenon of semi-immunity fuels the belief that it should, somehow, be possible to find a way to contain this disease through a vaccine.

Approaches have targeted all stages of the malaria life cycle (described in Section 1.2), which are roughly divided into pre-erythrocytic, targeting the sporozoites or the liver-stage; erythrocytic, targeting the asexual blood-stages; and

sexual stage targets. Alternatively, the vaccine candidates can be categorized as attempting a complete prevention of the disease, a reduction of the severity (morbidity and mortality), or as transmission-blocking. Yet another classification groups them into the use of whole sporozoites attenuated either chemically or by gamma irradiation, or by recombinant protein antigen [33].

Some vaccine candidates have simply failed in proof of concept. Other approaches have had backlashes due to ethical implications, such as genetically modifying mosquitoes to prevent their ability to transmit malaria parasites [43]. Similar issues around ethics were raised around the justification of injecting people with a transmission- though not disease-preventing vaccine (which is therefore considered to be a so-called altruistic vaccine) [44]. Yet other approaches have experienced a renaissance after years of dormancy when new techniques were developed, such as the initial approach of Nussenzweig et al. in 1967, who injected sporozoites attenuated through x-irradiation [45]. Only years later did Hoffman et al. manage to develop a manufacturing facility to produce these sporozoites safely, allowing this approach to restore hope among researchers and producing promising results to date [46–48].

Over 24 vaccine candidates are currently in the pipeline and in an advanced pre-clinical or clinical development stage, as depicted in the so-called "Rainbow Table" of the WHO, which was last updated July 17th, 2017 [1]. Figure 2 lists some of these candidates, grouping them into pre-erythrocytic, blood-stage, or transmission blocking candidates, as well as in the according research phases that they are currently in.

To elucidate all vaccine candidates would surpass the objective of this thesis, so only the most advanced vaccine candidate and the candidate with which this thesis is concerned will be illuminated in the following.

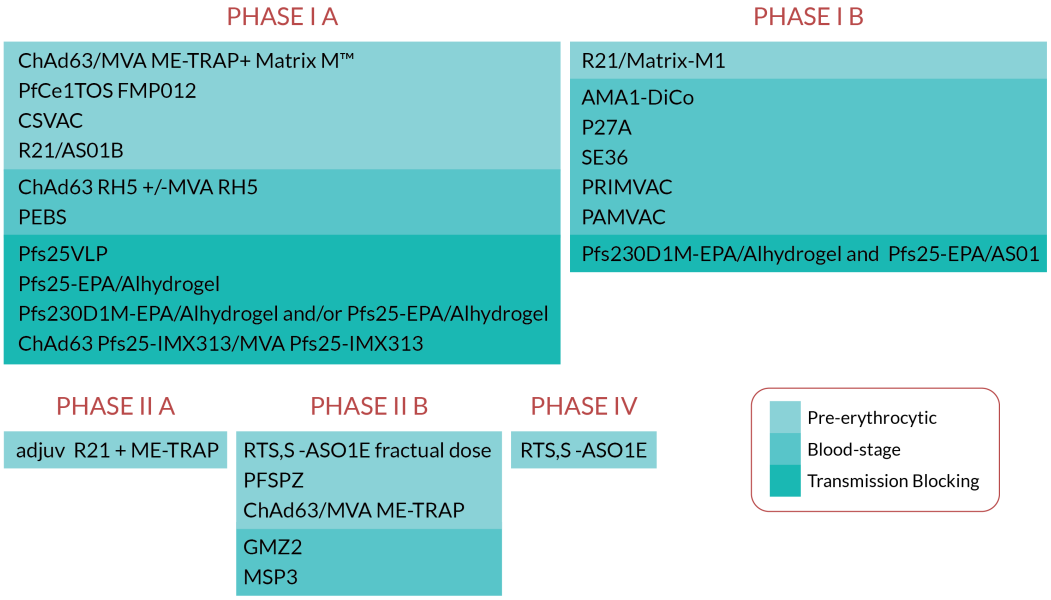

Figure 2: Global malaria vaccine pipeline, adapted from the WHO "Rainbow Table" [1], depicting the various project names for candidate vaccines and in which development phase they are, as well as which stage of the malaria life cycle they target.

1.7.1 RTS,S

The RTS,S (Mosquirix) malaria vaccine candidate is the one to which the media has given the most attention, and which has been most extensively tested among all malaria vaccine candidates. It received positive scientific evaluation by the European Medicines Agency (EMA) in 2015 [49], and in 2016 the WHO suggested implementation trials, which are underway in Ghana, Kenya, and Malawi since 2019 and will continue through 2024 [50, 51]. In October 2021, the WHO recommended the use of RTS,S in children aged 5 months or older, living in moderate and high malaria transmission regions, as the data from the implementation trials showed a favorable safety profile as well as proof that the vaccine could significantly reduce severe, life-threatening malaria cases [52].

The vaccine contains an antigen comprised of a monovalent recombinant protein, which targets a fragment of the surface protein on the sporozoite called the Circumsporozoite Protein (CSP), which is expressed on early liver forms of *P. falciparum* in the pre-erythrocytic stage of the parasite. The hepatitis B surface antigen is coexpressed with the CSP, and the most advanced vaccine candidate is formulated with AS01 as the adjuvant [53].

In a study published in 2014, the vaccine efficacy waned over a period of 18 months, starting out with reducing the incidence of clinical malaria by 47% during the first six months and declining to a reduction of 12% after 13-18 months [54]. The same study applied a booster vaccine after 20 months, increasing the vaccine efficacy compared to the prior mentioned results, but the overall results still leave room for improvement. In children aged 5-17 months, the vaccine efficacy measured by the number of clinical malaria episodes from month 0 to 12 months was 36.3% (versus 28.3% without the booster) [55]. In young infants, aged 6-12 weeks, the vaccine efficacy was 25.9% (versus 18.3% without the booster) [55].

Even with these modest results regarding vaccine efficacy, health economic calculations concluded that administering the RTS,S candidate vaccine would be a cost-effective measure. One study found that the vaccine intervention would be more cost-effective than providing insecticide-treated bed-nets in the case of Malawi, provided that the duration of effectiveness of the vaccine would be at least 2.69 years at a cost of less than $15, and bed net efficacy would be limited to less than 4.24 years [56]. Otherwise, bed nets win the race. Furthermore, a 2016 *Lancet* paper predicted "a significant public health impact and high cost-effectiveness of the RTS,S/AS01 vaccine across a wide range of settings", further underscoring the rationale for funders to continue supporting research and the implementation of this vaccine candidate [57].

1.7.2 Recombinant *Lactococcus lactis* Hybrid GLURP and MSP3 (GMZ2)

Blood-stage vaccine candidates usually target proteins on the surface of merozoites or parasite proteins on the surface membrane of infected erythrocytes. As one of the first blood stage vaccine candidates, GMZ2 has tried to induce a similar reaction as that seen in NAI, with the goal of parasite population reduction, rather than the goal of inducing sterile immunity. In doing so, it could provide a unique advantage to the aforementioned RTS,S vaccine candidate, as research suggests that the pre-erythrocytic target could merely shift the critical deadly episodes towards a later time in life, as no NAI would be developed.

As described in Section 1.6, there are a number of antigens that have been identified to promote NAI, such as MSP3 and GLURP. Furthermore, it was shown that when the levels of antibodies against both MSP3 and GLURP together were increased, the protection against clinical *P. falciparum* malaria was higher [58]. Following this, it was shown that the combination of the two in a recombinant fusion protein in the gram-positive bacterium *Lactococcus lactis* induced a stronger immune response than either one on its own, or even the two simply mixed together [59]. This led a team at the Statens Serum Institut in Copenhagen, Denmark, to look further into this opportunity for a potential vaccine candidate, referred to as GMZ2.

A first study to establish the aptitude of the vaccine candidate was performed on *Saimiri sciureus* monkeys, which concluded that partial protection against a malaria infection could be reached [60].

The safety of GMZ2 in humans was first assessed at the Institute of Tropical Medicine of the University of Tübingen, Germany, in a clinical phase I trial performed on 30 malaria-naive volunteers from 2006 to 2007 [61]. Three doses of either 10, 30, or 100 μg of GMZ2 adjuvanted with aluminum hydroxide were given. Along

with safety, immunogenic endpoints were determined as well. The study found all adverse reactions to have diminished within 24 hours and to have been of grade 2 or lower. Furthermore, a significant increase of GMZ2 antigen-specific antibodies was recorded after day 56 and day 84, as well as after one year of vaccination [61].

This led to a second phase I clinical trial, testing the safety and immunogenicity in 40 healthy, semi-immune Gabonese adults at the Medical Research Unit in Lambaréné, Gabon (Centre de Recherches Médicales de Lambaréné (CERMEL)), from 2007 to 2008 [62]. Half the volunteers received three doses of 100 μg of GMZ2 adjuvanted with aluminum hydroxide, compared to a control group, which received rabies vaccine dosages. The results showed the vaccine candidate to be safe and immunogenic, with similar response-patterns found in the aforementioned study.

A multicenter phase IIb clinical trial followed between 2010 to 2011, which assessed the efficacy of GMZ2 adjuvanted in aluminum hydroxide in 1,849 12-60-month-old African children. The results, adjusted for age and site, showed a vaccine efficacy of 14% [63]. From a cost-effective viewpoint, these results would not satisfy the international public health expectations for widespread roll-out, so the paper called for studies to improve immunogenicity.

As we know that different adjuvants can increase the efficacy of vaccines, the Statens Serum Institut combined GMZ2 with the novel and more potent adjuvant Cationic Adjuvant Formulation 01 (CAF01), which the aforementioned institute had developed. This was chosen due to results from multiple clinical trials which demonstrated that CAF01 showed positive effects with regards to both safety and immunogenicity in different vaccines tested [64, 65], most prominently with a tuberculosis vaccine [66]. CAF01 is a "two-component liposomal adjuvant system composed of a cationic liposome vehicle (dimethyldioctadecyl-ammonium (DDA))

stabilized with a glycolipid immunomodulator (trehalose 6,6-dibehenate (TDB))" [66].

This study was the first trial of GMZ2 adjuvanted with CAF01 in healthy adult volunteers, followed by Controlled Human Malaria Infection (CHMI), as described in the following section, to assess the efficacy of this new candidate vaccine.

1.8 Evaluating Malaria Vaccine Candidate Efficacy Using CHMI

Microbial challenges in human beings with microorganisms have been performed for over 200 years, with Edward Jenner being the first recorded example of variolating a child with smallpox in order to prove the efficacy of his vaccine [67]. Julius Wagner-Jauregg infected patients suffering from general paralysis with malaria (*P. vivax*) but also with tuberculin, typhoid or streptococci in 1917 as a means to treat them with the caused fever [68, 69]. The application of *P. vivax* on patients with neurosyphilis later on won him the Nobel Prize in 1927, and the fever therapy was used until the 1950s [70].

Controlled human infection studies can provide insights into the biological mechanisms of how an infection takes place, as well as the pathways of host-pathogen interactions, but they can also provide insights into the efficacy of new treatments or vaccines. However, human challenges are also associated with horrific scientific experiments, including those during the Nazi regime [71], which have been the motivation for the current scientific community to ensure that ethically justifiable and sound methods are developed instead. As opposed to prior practice, a prerequisite for the rationale behind modern human challenges is that the selected disease is safely treatable or self-limiting, causing no lasting damage to the participant. Nev-

ertheless, the principle set by Hippocrates to "first do no harm" is, by definition, impossible to adhere to, as an infection obviously must take place. The question always needs to be posed whether the risk and burden on the volunteers, as well as on third parties (for example, through the potential transmission from a volunteer) are justifiable, and whether these factors are outweighed by the potential benefit that the study brings. Further ethical questions regarding the remuneration of volunteers (to not create financial incentives) and how to select or exclude participants (to guarantee informed consent) are still being discussed [72, 73]. Provided that the investigator is properly trained according to international standards and protocol, with the highest priority being set on the participants' health and safety, then the method of human challenges can be seen as ethically sound, under the condition that the trial aims to address a greater societal problem, such as the acceleration of the development of novel interventions in order to reduce the disease burden on the affected population [74]. Ethical principles, such as those set by the Declaration of Helsinki, and general standards, such as The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) standards, should obviously be followed as well while conducting human trials.

A large contributor to the slow progression of developing a malaria vaccine could be attributed to the costly and time consuming nature of traditional field trials, as large study groups and sites with high malaria prevalence are required. The methodology of human challenges has expedited this process by allowing direct informed consent and small sample sizes, as well as avoiding the risk of including non-exposed controls through the ability to infect candidates at a near 100% probability, as opposed to the distinction of protection from non-exposure in the field, which is not possible. This enables the sieving out of inadequate vaccine candidates at an early stage, and therefore also averts expensive trials [75]. Therefore,

since the late 1980s, scientists have reared infected mosquitoes, which they then placed on a participant's skin patch in a controlled manner to try to guarantee a malaria infection in the person [76]. For this, about 4-7 mosquito bites had to take place [77]. 1,343 participants are said to have taken part in CHMI between 1985-2009, and the procedure has been reported to be safe, with only one cardiac complication having been reported, of which the causality to the CHMI could not be verified [76, 78].

As the method using mosquito bites requires high biosafety level insectary facilities, a more convenient way of infection via direct intradermal or intramuscular injection was developed [79, 80]. Furthermore, mosquito-mediated CHMI was difficult to standardize, as the currently available protocols use *A. stephensi* as a vector, which is a mosquito widely present in India. Therefore, it should not be imported to the African continent, as it is a good vector that could accidentally spread. The usage of *Plasmodium falciparum* sporozoites (PfSPZ) does not have this problem. Trials such as one performed in Tanzania [81], one in Gabon [82], and two performed in Kenya [83, 84] proved the applicability in volunteers living in a malaria endemic setting and noted unique, yet conquerable, challenges from which future studies could learn. Due to a large amount of research being conducted in high-income countries, most human challenges have been done on volunteers from high-income countries, although the pathogens the challenges were conducted with are primarily diseases endemic to low and middle income countries. The consequence of this could be that the generalizability of the results might not be guaranteed for the target population in the relevant regions. All the aforementioned studies aimed to have volunteers included in their studies who would constitute a representative sample of the malaria-affected population as regards characteristics such as genetic traits or microbiomes [72]. The procedure was further optimized at the University of Tübingen, by means of direct venous inoculation (DVI) of 3,200 PfSPZ, which

resulted in a 100% infection rate in the participants [85].

In this study, the newly standardized CHMI method allowed an assessment of the efficacy of GMZ2CAF01, making this the first time that CHMI was used for a study on an asexual blood-stage malaria vaccine candidate.

1.9 Study Objectives and Endpoints

1.9.1 Objectives

The primary objective of the present study was to determine a regimen of GMZ2-CAF01 that would successfully reduce parasite multiplication. This was measured through the method of CHMI, performed through DVI as described in Section 1.8, and quantified through the measurement of time to the onset of malaria symptoms, parasitemia, and parasite kinetics. To attain these factors, the volunteers were clinically assessed, thick blood smears (TBS) were analyzed under a microscope, and quantitative Real-Time Polymerase Chain Reaction (qPCR) was obtained.

Furthermore, the objective was to evaluate the safety of CHMI as a tool in malaria research in an African setting. For this, the participants were closely monitored for adverse event (AE)s during the procedure of the CHMI.

1.9.2 Endpoints

The primary endpoint of this study was the time taken to reach malaria due to the CHMI, defined by the finding of asexual blood stage *P. falciparum* parasitemia detected by microscopy, joined with at least one symptom typical for malaria.

The secondary endpoint of this study with respect to efficacy was the time until the development of asexual blood stage parasitemia due to the CHMI.

Furthermore, the number or occurrence of at least possibly related AEs or serious adverse event (SAE)s from the time of the CHMI until the end of the follow-up period was an endpoint.

2 Methods

2.1 Study Design and Site Description

2.1.1 Study Design

The study was performed between April and December of 2015 at the CERMEL in Gabon. The protocol called for 50 participants to be enrolled, as further described in Section 2.2. The study was a double-blind, randomized, controlled, single-center phase I clinical trial and was approved by the National Ethics Committee of Gabon, as well as the Gabonese Ministry of Health, and was financed by the German Ministry of Education and Research (BMBF) and the German Center for Infection Research (DZIF). The study was performed according to the guidelines of ICH and the GCP guidelines, as well as those of the Declaration of Helsinki. The study was registered with the Pan-African Clinical Trials Registry under the trial number: PACTR201503001038304. Volunteer safety was guaranteed through constant monitoring by local safety and scientific committees.

The participants were divided into Groups A, B, C, D, and E, with 8, 12, 8, 12, and 10 participants per group, respectively. Group A was the control group and received three rabies shots (Rabipur) and the CHMI challenge. Group B received 100 μg of the GMZ2 vaccine candidate adjuvanted with aluminum hydroxide suspension (Alhydrogel) at a concentration of 0.85 mg aluminum per dose and received the CHMI challenge. Group C received 30 μg of GMZ2 adjuvanted with CAF01 and received the CHMI challenge. Group D received 100 μg of GMZ2CAF01 and the CHMI challenge. Lastly, Group E received 100 μg of GMZ2 adjuvanted with CAF01, but did not receive the CHMI challenge. Table 1 depicts the five arms of the study, as described above.

Table 1: Five arms of the study depicting the number of participants in each group as well as which vaccine regime they received and whether they subsequently received Controlled Human Malaria Infection (CHMI). GMZ2 = Recombinant *Lactococcus lactis* Hybrid GLURP and MSP3. GLURP = Glutamate Rich Protein. MSP3 = Merozoite Surface Protein 3. CAF01 = Cationic Adjuvant Formulation 01. PfSPZ = *Plasmodium falciparum* sporozoites. Alum = aluminium hydroxide suspension (Alhydrogel).

	Group	Number of Participants	Immunization	CHMI (3200 PfSPZ)
	A	8	3 x Rabies	✓
	B	12	3 x 100µg GMZ2 in Alum	✓
	C	8	3 x 30µg GMZ2 in CAF01	✓
	D	12	3 x 100µg GMZ2 in CAF01	✓
	E	10	3 x 100µg GMZ2 in CAF01	
Total		50		

2.1.2 Site Description

The study was conducted at the CERMEL, which is located in Lambaréné, Gabon, next to the Albert Schweitzer Hospital, which was founded by Albert Schweitzer in 1913. The predecessor of CERMEL, the medical research unit, was established in 1981 and has since conducted numerous clinical trials, such as the prior GMZ2 study mentioned in Section 1.7.2.

In 2015, the year in which the study was performed, Gabon had 217,287 presumed and confirmed malaria cases, representing 12.59% of its entire population of 1,725,292 people, as estimated by the United Nations (2015) [3].

Lambaréné is the capital of the Moyen-Ogooué region, with a population of 38,775 as of 2013 [86]. The area is graded as hyperendemic concerning malaria trans-

mission, with *Anopheles gambiae* and *Anopheles funestus* being the main vectors, although the ultimate area around the Albert Schweitzer hospital seems to have enjoyed a reduced rate of parasites due to advanced sanitation and access to health care [87].

2.2 Selection of Participants and Contra-Indication to Vaccine Administration

The study was performed on 50 volunteers, who had to fit the following criteria to minimize the risks of potential adverse events.

2.2.1 Inclusion Criteria

The study protocol called for healthy male adults between the age of 18 to 40 years, with a body mass index (BMI) of less than 35, and a life-long history of exposure to malaria, specifically from areas of high transmission of *P. falciparum* malaria. They had to live in Lambaréné, be willing and able to comply with the study requirements, and be reachable by mobile phone at all times. Furthermore, the participants had to be willing to receive two anti-malaria regimes and refrain from donating blood during the time of the study. A written informed consent form had to be filled out, and a quiz on the procedure and risks that came with participating in this study had to be answered correctly.

2.2.2 Exclusion Criteria

The study protocol excluded participants who had already taken part in a malaria vaccine investigation at any point of their life, or had received any investigative

product in the past 30 days prior to the start of their participation in this study. The participants were also excluded if they currently had malaria, hepatitis B and/or hepatitis C, were HIV-positive or had any other immunosuppressive condition, had any severe or chronic infections, sickle cell disease or other blood diseases, cancer, psychological conditions, or history of seizure. Electrocardiogram (ECG), blood and urine tests, as well as clinical examinations were not to show any abnormal results. Alcohol and/or drug abuse also excluded them from the study, as well as any indication that closely following them would not be possible. They were not to have been immunized with four or more other vaccines within the past month, nor have received any blood products or immunoglobulins within the past three months prior to the start of the study. Participants were further excluded if they showed any indications of allergy to any of the products used in the experiment, including the antimalarial treatments used or past vaccine reactions.

2.2.3 Withdrawal

The participants were able to withdraw from the study at any time, without having to give any reason for their withdrawal, and investigators were encouraged to withdraw any participant as soon as there was any indication that a withdrawal would be beneficial to the participants health and well-being in any way. Furthermore, participants were withdrawn as soon as any of the ineligibility criteria were met, the participant showed non-compliance, if there was a significant deviation from the protocol, or the investigator decided this for administrative reasons. Another withdrawal reason was the occurrence of any AE that required the participant to refrain from continuing in the study.

2.2.4 Criteria to Stop Vaccination

Certain criteria would have led to the halt of the vaccination procedures, should they have occurred during the trial. However, the follow-up would have been continued in these cases in order to assess for AEs and safety data. The criteria to stop vaccination were: the presentation of an acute allergic reaction, defined as significant IgE-mediated events, as well as any anaphylaxis post-vaccination; significant illness, such as one indicated by a body-temperature above 38 °C for more than 14 days post-vaccination; the use of any other experimental drug, vaccine or substance other than the ones administered in this study within the duration of the study, as well as the use of any blood products or immunoglobulins. Furthermore, the administration of immunosuppresants or other immune-altering drugs for over 14 days during the study period would have been a criterion to stop the vaccination, with the exception of daily inhaled or topically administered steroids of up to 0.5 mg/kg. Lastly, the incorrect administration of the study vaccine would have also led to the stop of any further vaccinations.

2.2.5 Criteria to Delay Vaccination and CHMI

The schedule of the vaccination and CHMI could have been delayed up to 14 days if an acute disease occurred on the day of either vaccination or CHMI. However, mild symptoms such as diarrhea or mild upper respiratory infections without fever were no criteria to stop the vaccination or CHMI.

2.3 Study Procedures

2.3.1 Screening and Randomization

First, the screening was completed and the eligible participants were thoroughly interviewed and informed about the study. Medical parameters were measured and recorded. The biochemistry parameters were creatinine, AST (aspartate transaminase), and ALT (alanine transaminase) which were measured by Cobas Mira Plus (Roche, Basel, Switzerland). The hematology parameters that were measured were the erythrocyte number, the hemoglobin concentration, hematocrit, platelet count, as well as the differential and total leukocyte count, all of which were measured by ABX Penta 60 (Horiba, Kyoto, Japan). A study identification number (ID) was assigned to all of the included participants, and a computer-generated, randomized list was obtained to allocate the participants to one of the five arms of the study (Groups A - E, see Table 1).

2.3.2 Vaccination

Between April 20th and June 18th, 2015, the participants received intramuscular vaccine injections into their deltoid muscle, alternating sides for the three injections, with four weeks between each injection and thorough monitoring after each individual vaccination was performed and any AEs recorded. Group A received the rabies vaccination, whereas Groups B - E received the candidate vaccine. As the different formulations had visible different appearances, the study team performing the task of the vaccination was excluded from continuing any work on the study until the allocated treatment arms were unblinded. The participants were observed intensely for AEs during the following 14 days, and intermittently the following six months.

2.3.3 CHMI

93 to 97 days after the last vaccine injection, so from September 18th until 21st, 2015, the participants of Group A - D were regrouped into Groups 1 to 3, to achieve a randomization for the time of the CHMI independent of the vaccination group. Following this, they received 300 mg clindamycin twice a day for five days in order to clear any potential malaria infection. Clindamycin has been shown to be highly efficacious against asexual liver and blood stage parasites and is an antimalarial regimen that has proven to be well tolerated [88]. Before iatrogenic infection, it was further checked through microscopy of TBS that the participants had currently no *Plasmodium* parasitemia. Three days later followed the CHMI via DVI with 3,200 aseptic, purified, vialed, cryopreserved, infectious PfSPZ, strain NF54, which were kindly produced and shipped to Gabon in liquid nitrogen vapor phase by Sanaria Inc., Rockville, MD, USA. As the half-life of Clindamycin is 2 to 4 hours [89], there was no risk of this treatment interfering with the CHMI as there were three days between the last dose of Clindamycin and the CHMI. Close observations of the participants for AEs were made for the next five days, as further defined in Section 2.4.

2.3.4 Outcomes and Follow-Up

TBS, 1 ml blood samples for qPCR, as well as clinical signs and symptoms, were collected every day post-CHMI until day 35, or until malaria was diagnosed. As soon as malaria was diagnosed, or after day 35 after the CHMI (C+35), the participants received artemether-lumefantrine (80mg/480mg) at the following time-points: 0, 8, 24, 36, 48, and 60 hours. The volunteers were seen as cured after negative TBSs were found on two consecutive days. Figure 3 shows the timeline as described above, furthermore dividing the timeline into the 3 random groups that

Location	Time Point Coding
Either	B 1
Either	B 2
Either	B 3
Either	B 4
Either	B 5
CERMEL	C -7
CERMEL	C -6
CERMEL	C -5
CERMEL	C -4
CERMEL	C -3
Either	C -2
CERMEL	C -1
CERMEL	C0
CERMEL	C +1
Home	C +2
Home	C +3
Home	C +4
Home	C +5
CERMEL	C + 6 to 34/Malaria
CERMEL	Until C+34 OR day of malaria

Date	Group 1	Group 2	Group 3
6-Sep	B1		
7-Sep	B2	B1	
8-Sep	B3	B2	B1
9-Sep	B4	B3	B2
10-Sep	B5	B4	B3
11-Sep	C -7	B5	B4
12-Sep	C -6	C -7	B5
13-Sep	C -5	C -6	C -7
14-Sep	C -4	C -5	C -6
15-Sep	C -3	C -4	C -5
16-Sep	C-2	C -3	C -4
17-Sep	C-1	C-2	C -3
18-Sep	C0	C-1	C-2
19-Sep	C+1	C0	C-1
20-Sep	C+2	C+1	C0
21-Sep	C+3	C+2	C+1
22-Sep	C+4	C+3	C+2
23-Sep	C+5	C+4	C+3
24-Sep	C+6	C+5	C+4
25-Sep	C+7	C+6	C+5
26-Sep	C+8	C+7	C+6
27-Sep	C+9	C+8	C+7
28-Sep	...	C+9	C+8
29-Sep	...	...	C+9
...	Until C+34 OR Day of Malaria		

C = Challenge

Clindamycin

Challenge

TBS, Physical Checkup

Figure 3: Timeline of the study showing the different time points (B is before the treatment, C stands for the time of the challenge, and the number gives the days before or after the event), when clindamycin was given, when the CHMI challenge was performed, and during what times follow-up was done. Furthermore it shows the location where follow-ups were done (at home or at the research center (CERMEL), or if either location was fine). Participants were randomly allocated to Groups 1 - 3.

the participants were put into (Group 1-3). Time points B1 until B5 are the days before the treatment with clindamycin. Time points C-7 until C-3 (so 7 days before CHMI until 3 days before CHMI) are the 5 days in which the participants received clindamycin, as depicted by the light orange shading. Time point C0 is the day of the CHMI, after which the participants had to return to CERMEL the next day (C+1) to be checked up. During time points C+2 until C+5 the participants did not have to come into CERMEL, as it is known that it is extremely unlikely to already have an infection happening this short after the challenge. However, from day 6 (C+6) onwards the participants had to come to the CERMEL every day to ensure maximum safety precautions, as this was the time a potential infection was most likely to occur.

The patients lost to follow-up were given an antimalarial scheme and thoroughly checked and interviewed, and all the findings were recorded.

2.3.5 Malaria Diagnostic Procedure

There are different methods used to determine parasite count with microscopy. As the WHO method [90, 91] has shown to have a lower validity than the Lambaréné method (later also published by Mischlinger et al. [92]), this study chose to use the latter as the preferred method. For this method, exactly 10 μL of blood are placed on a 10 x 18 mm area on a microscopy slide, dried, then stained with 20% Giemsa in buffered water at pH 7.1 for 20 minutes, rinsed with water, air-dried, and then read by a trained malaria microscopist. For this, the microscopist counts the parasites on a light-optical microscope at a 1000x magnification level and divides this number by the high power fields (HPF)s, and lastly, multiplies this by the microscope factor [93, 94]. The microscope factor can either be calculated or measured; it gives the number of HPFs that need to be read in order to cover 1 μL

of blood on a TBS. This calculation via the Lambaréné method gives the number of parasites per μL of blood.

Figure 4 depicts the steps, that were taken each time a participant came to CERMEL during the specific time points as listed in Figure 3. The participants came in, samples were taken and slides prepared, which were then stained and dried. The slides would then arrive at the clinical laboratory where the readings were coordinated. In case a slide was found positive for plasmodia, a parasitemia result was calculated and immediately reported to the study physician, who would take necessary action to provide treatment to the patient.

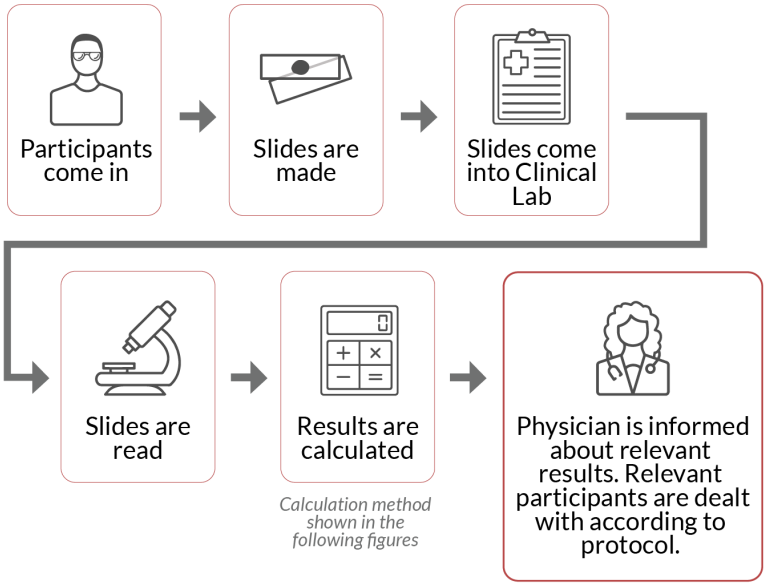

Figure 4: Workflow procedure at CERMEL, showing what measures were taken once the participants came in to the lab.

Figure 5 shows the triage of the days C+1 until C+35, or until the day of malaria. If the participants came to CERMEL at the according times and as communicated to them, they were examined and asked for symptoms, while the TBS was produced from the blood sample and read immediately by a team of qualified

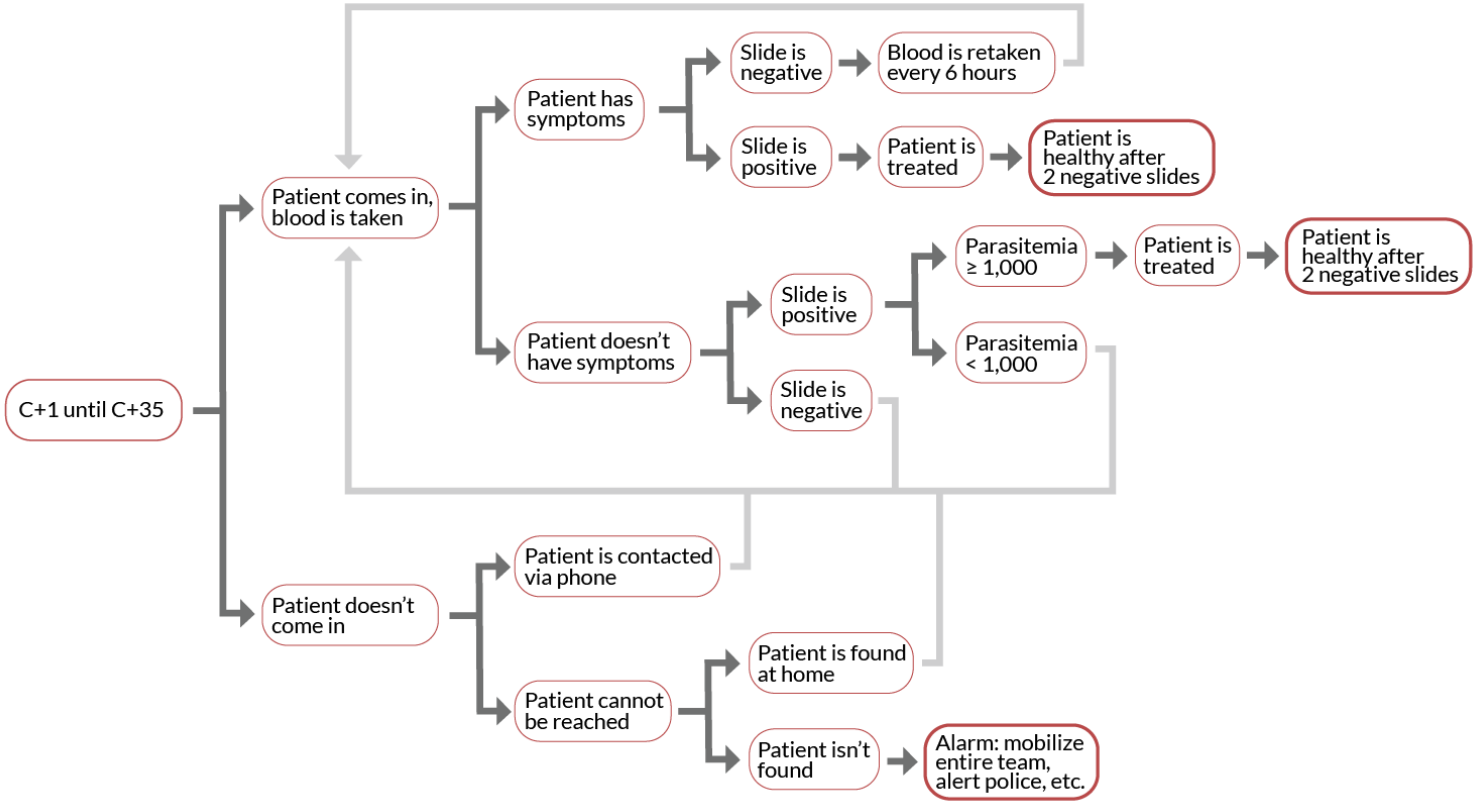

Figure 5: Workflow depicting actions taken depending on malaria slide results and patient symptoms. C+1 until C+35 are the days post Controlled Human Malaria Infection. The decision tree repeats until the patient is treated and has 2 negative slides, or after C+35, after which he is also treated with artemether-lumefantrine.

microscopists.

If the participant had symptoms but the slide was negative, a TBS was redone every 6 hours. If the slide was positive, the participant was treated and declared as healthy after two negative slides on two consecutive days. If the participant did not have symptoms but the slide was positive, then the participant was only treated if the parasitemia was above 1,000 parasites per μL of blood. This way it was possible to make a judgement on the parasite kinetics in participants who were able to control the infection.

If, however, the participant did not come to CERMEL at the according times as originally agreed with them, then they were first tried to be reached by phone. If this was not possible, a field visitor would try to find them at or near their home. If they would have still not been found, then the entire team would have been mobilized, as well as the local police in order to find the participant as soon as possible.

Figure 6 shows how the malaria slide readings were calculated. Two readings were done by two different microscopists. If, for example, both found 0 malaria parasites, a slide was redone after 24 hours. If both readers had a result that was equal to or above 300, then the mean was taken of both readings, provided the discrepancy in their ratio was not 2 or above 2. If that mean was 1,000 or above, then the participant was treated. If it was below 1,000, then the participant was only treated if he had symptoms. If, however, the discrepancy in their ratio was above 2, then a third reading was needed. For all scenarios, see Figure 6.

Furthermore, Figure 7 shows some examples with concrete reading results to clarify how readings were calculated in different scenarios, and what the according result would have been.

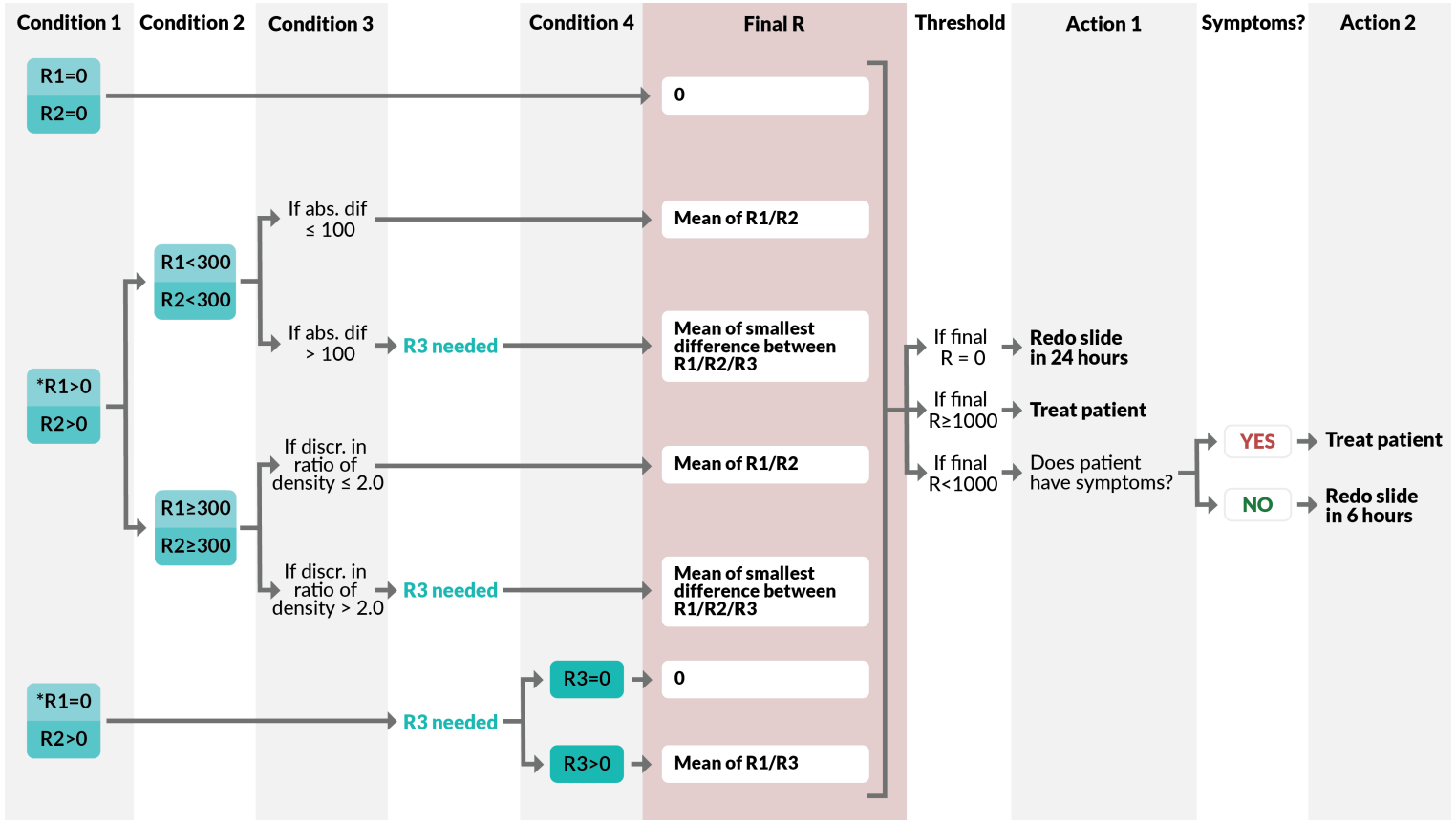

Figure 6: Malaria slide reading calculation decision tree, showing which action is to be taken under which conditions. R1 refers to Reading 1, R2 to Reading 2, R3 to Reading 3, Final R refers to the final reading result. The asterisk indicated that the condition also applies in the reverse. The mean refers to the geometric mean.

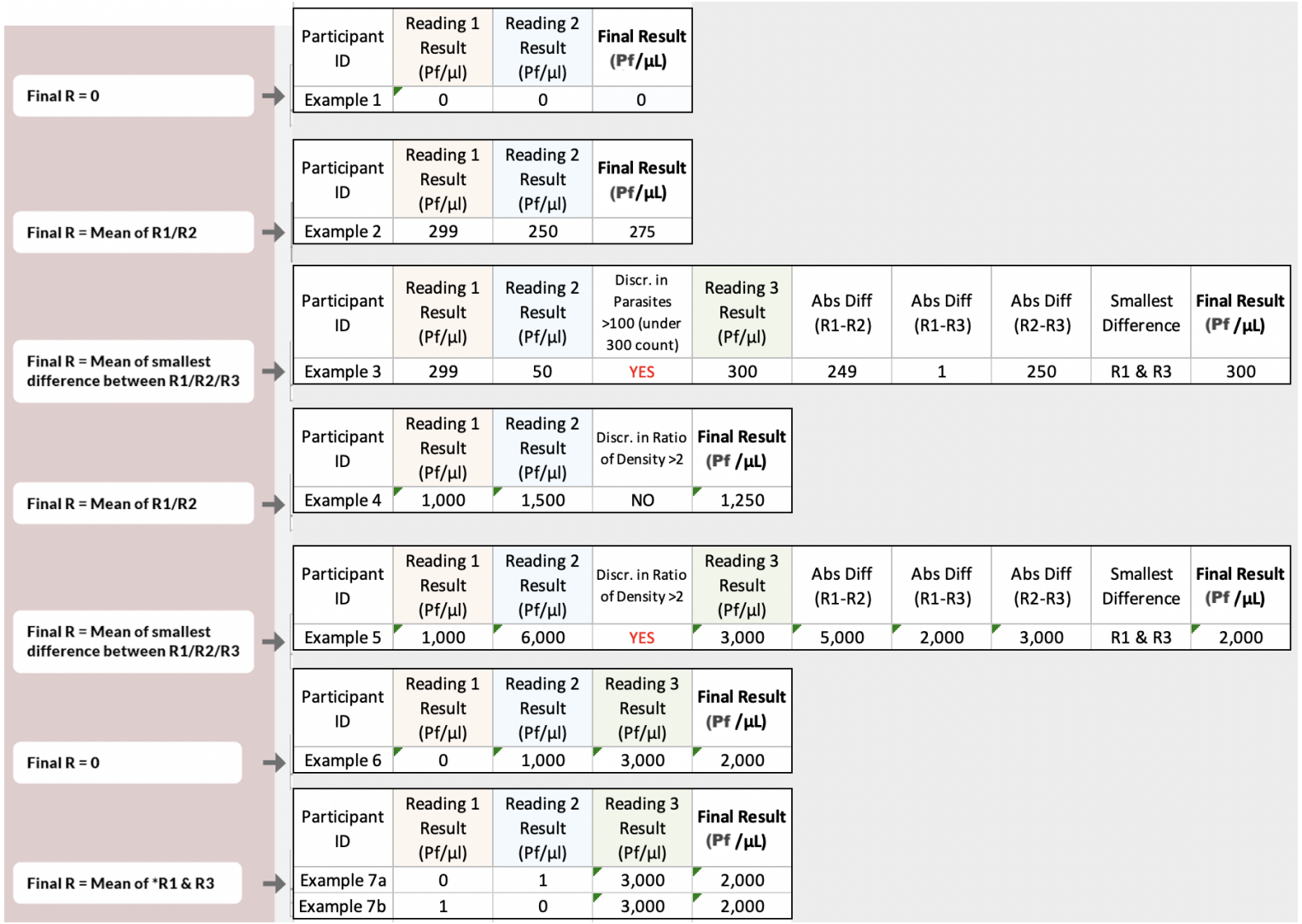

Figure 7: Examples of reading calculation scenarios. R refers to the Reading result of the malaria slide, R1 refers to Reading 1, R2 to Reading 2, R3 to Reading 3, Final R refers to the final reading result. Pf = *Plasmodium falciparum* parasites.

2.4 Adverse Events and Reporting Procedures

AEs were defined as any unexpected medical incident that a participant had at any time from the point of inclusion until the end of the trial that could temporarily be associated with the application of a medicinal product. The AEs of special interest, in this case, were the infection with malaria after the vaccination. Any AE, whether possibly related to the usage of a vaccination or not, that was recorded after the first immunization and four weeks after the last immunization was further specified as an AE following immunization (AEFI). On the other hand, SAEs were defined as incidents that were life-threatening, required inpatient hospitalization, resulted in significant disability, or resulted in death.

Malaria diagnosis was defined by the finding of asexual blood stage *P. falciparum* parasitemia detected by microscopy, joined with at least one symptom typical for malaria.

The safety was reported by monitoring any occurrence of AEs and SAEs that were in relationship with the CHMI. This was monitored by keeping the volunteers at the clinic for at least 30 minutes after the CHMI. Furthermore, following the CHMI, the volunteers were checked on at least once daily post-CHMI, but 2 to 3 times daily from day 6 post-CHMI onwards until the presentation of malaria, or until day 35. During all visits, open questions were posed to the volunteers, alongside questions regarding local and systemic symptoms, to ensure that all possibly related events were accurately surveyed. Everything was done to ensure that the contact with the volunteer remained reliable and constant during the entire period of the trial.

2.5 Statistical Considerations

The sample size was chosen in order to make a judgment about very frequent tolerability issues as well as safety issues. The time from CHMI to malaria was calculated using the Kaplan-Meier method to build survival curves, and differences between the groups were assessed by the log-rank test. Cox proportional hazard models were used to evaluate the relationship between the different candidate vaccines and the time to malaria. A Forest plot was used to represent the relationship between the different candidate vaccines and time to malaria in each group. A two-tailed type I error P-value of <0.05 was considered statistically significant. The data was analyzed with R Version 1.3.959, with graphics being produced using the package ggplot2, along with packages tidyverse, survival, and survminer.

3 Results

3.1 Participant Flow

As visualized in Figure 8, a total of 91 volunteers were assessed for eligibility to participate in the study. Of these, 41 were excluded from the study: 16 participants did not meet the criteria as described in Section 2.2, and six participants retracted their consent to participate in the study. Of the remaining 69 eligible volunteers, no more than 50 were needed as called for in the protocol, therefore excluding another 19 participants from the study. The 50 participants were then randomly allocated into the five groups, as shown in Table 1, to receive the three consecutive vaccine injections.

Of Group A, all eight volunteers received the first vaccination, seven received the second shot, and six remained to receive the third vaccination. Of Group B, all 12 participants received all three vaccinations; of Group C, all eight participants received all three vaccinations; and of Group D, all 12 volunteers received all three vaccinations. Of Group E, all 10 volunteers received the first vaccination, and nine received the second and third vaccination. Groups A, B, C, and D then received CHMI 93 to 97 days after the last injection. However, one participant dropped out after the third vaccination and before CHMI in each of the Groups A, B, and D, respectively. Therefore, five participants received CHMI in Group A; 11 participants in Group B, and 11 participants in Group D.

3.2 Losses and Exclusion

Three participants did not complete the vaccination schedule because they relocated to another town. Two of these participants were from Group A, and one

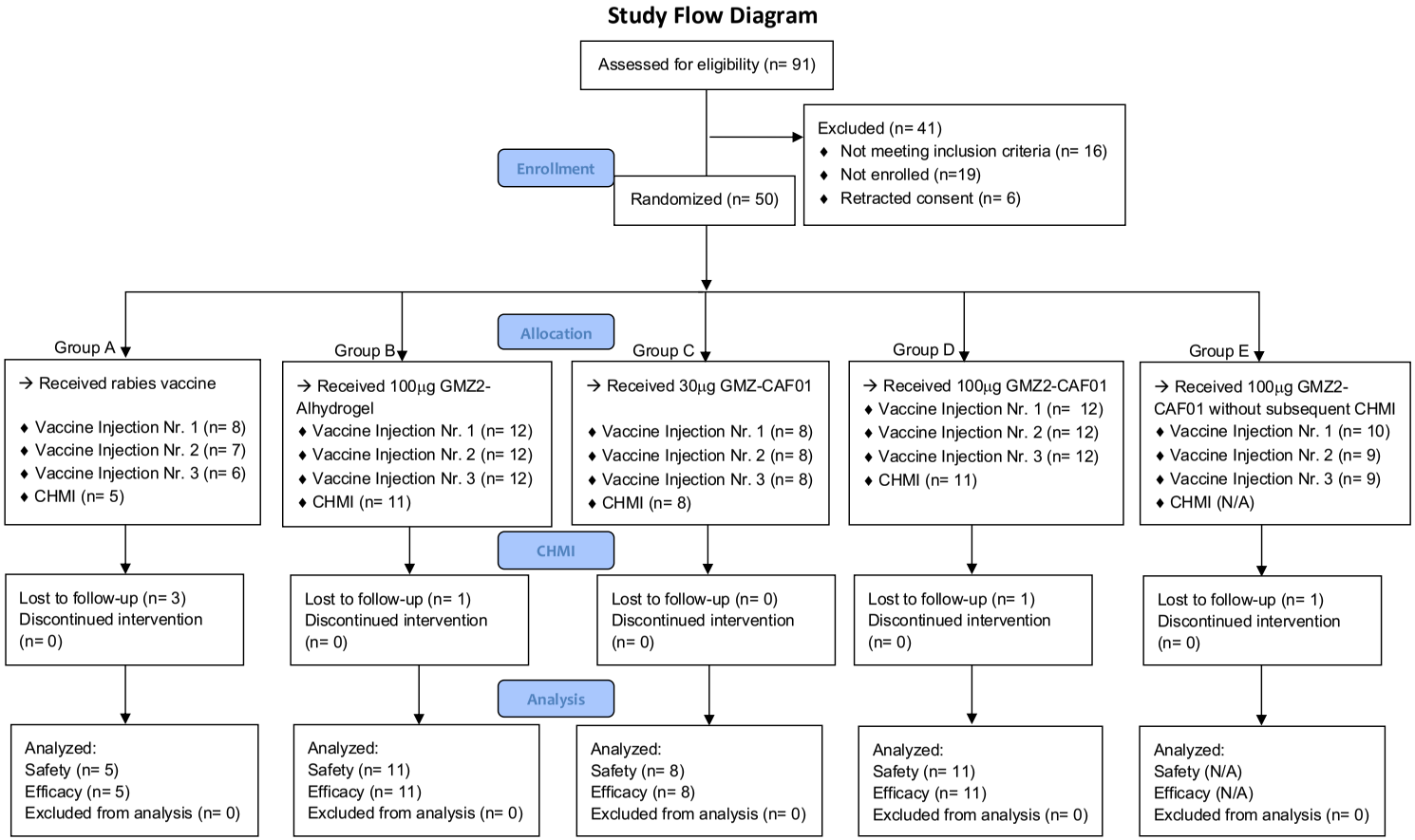

Figure 8: Participant Flow Diagram

participant was from Group E. After this, another three participants (one from each Group A, Group B, and Group D, respectively) dropped out because they had relocated to another town, and therefore could not participate in CHMI.

After CHMI, one volunteer from Group B was lost due to a relocation to Cameroon. This participant was therefore treated with artemether-lumefantrine on day 14 to 16 post-CHMI, as called for by the protocol, even though he did not develop microscopically detectable parasitemia.

3.3 Baseline Demographics

As summarized in Table 2, the age of the participants ranged from 18.1 to 37.4 years, and the BMI ranged from 16.7 to 29.7 kg/m^2. All of them had spent their entire lives in Lambaréné, which is a place of high transmission of *P. falciparum*, as called for by the protocol.

3.4 Numbers Analyzed

As summarized in Figure 8, five participants were analyzed for safety and five for efficacy in Group A, 11 participants were analyzed for safety and 11 for efficacy in Group B, all eight participants were analyzed for both safety and efficacy in Group C, and 11 participants were analyzed for safety and 11 for efficacy in Group D.

Table 2: Baseline demographic data for each group, showing the distribution of age and BMI (body-mass-index) over the 5 groups (Groups A-E).

Group	Median Age (in years)	Minimum Age (in years)	Maximum Age (in years)	Median BMI (in kg/m^2)	Minimum BMI (in kg/m^2)	Maximum BMI (in kg/m^2)
A n = 8	23.75	21.8	35.5	23.30	16.7	25.3
B n = 12	24.40	19.2	32.2	22.10	18.8	29.7
C n = 8	22.50	20.3	35.1	21.65	19.1	23.1
D n = 12	21.75	18.1	34.0	21.45	18.8	24.1
E n = 10	21.50	18.2	37.4	21.65	18.8	25.6

3.5 Outcomes and Estimates

3.5.1 CHMI Safety

Overall, there were no SAEs during the entire course of the study [95]. However, of the participants who received CHMI, all had at least one AE with the exception of three participants – two of them were in Group C, and one in Group D. One participant without AEs in Group C had protection from malaria and the other one had low oscillating parasitemia with no symptoms. The participant with no AEs in Group D had malaria, which in this case was of course not defined as parasitemia plus at least one symptom but rather defined as having a parasitemia of over 1,000. Of the 174 AEs that occurred during CHMI, 161 were Grade 1 AEs and 13 Grade 2 AEs. 35 AEs could at least possibly be related to the study, of which three participants had Grade 2 AEs. Of these, one had pyrexia, which occurred in

Group A to a participant who had malaria; one participant in Group B who had malaria had Grade 2 headache as well as Grade 2 myalgia; and one participant from Group C who also had malaria had Grade 2 fatigue. A summary of all the AEs that could at least possibly be related to the study is shown in Tables 3, 4, and 5, using the preferred Lowest Level Terms of the Medical Dictionary for Regulatory Activities (MedDRA) [96].

The most common possibly related AEs due to CHMI was Grade 1 headache (seven times, experienced by six participants, all of whom had malaria at the time of the occurrence of the AE) and nausea (five times, experienced by five participants, also all of whom had malaria).

Table 3: Distribution of Grade 1 AEs (adverse events) among the participants who had malaria. The AE terms were chosen from the Medical Dictionary for Regulatory Activities (MedDRA). The number indicates the amount of times the AE was recorded. One asterisk indicates that 2 participants had this AE (so one participant had the AE twice); two asterisks indicate that 6 participants had this AE (so one participant had the AE twice).

Grade 1 Adverse Events

MedDRA Term	Malaria
Arthralgia	3
Chills	1
Diarrhea	3*
Fatigue	4
Feeling cold	2
Headache	7**
Injection site pain	0
Myalgia	1
Nausea	5
Pyrexia	2

Table 4: Distribution of Grade 1 AEs (adverse events) among the participants who had oscillating parasitemia. The AE terms were chosen from the Medical Dictionary for Regulatory Activities (MedDRA). The number indicates the amount of times the AE was recorded.

Grade 1 Adverse Events

MedDRA Term	Control
Diarrhea	1
Fatigue	1
Injection site pain	1

Table 5: Distribution of Grade 2 AEs (adverse events) among the participants who had malaria. The AE terms were chosen from the Medical Dictionary for Regulatory Activities (MedDRA). The number indicates the amount of times the AE was recorded.

Grade 2 Adverse Events

MedDRA Term	Malaria
Fatigue	1
Headache	1
Myalgia	1
Pyrexia	1

3.5.2 Vaccine Efficacy

As depicted in Figure 8, a total of 35 volunteers received CHMI, of which one volunteer dropped out of the study during this phase (see Section 3.2) and treated prematurely. The remaining 34 were monitored on a daily basis, of which 15 were diagnosed with malaria and subsequently treated with artemether-lumefantrine. Of these 15 volunteers, two were in Group A, six in Group B, two in Group C, and five in Group D (see Table 6). Five participants did not develop malaria, of which one was in Group A, one in Group B, two in Group C, and one in Group D. 14 participants developed low oscillating parasitemia but showed no symptoms, which are summarized in Table 6.

Table 6: Distribution of the number of participants who developed malaria, the number of participants who developed no malaria, and the number of participants who had low oscillating parasitemia per Group.

	Group A	Group B	Group C	Group D
Developed Malaria	2 of 5 participants (40%)	6 of 10 participants (60%)	2 of 8 participants (25%)	5 of 11 participants (45.5%)
Developed No Malaria	1 of 5 participants (20%)	1 of 10 participants (10%)	2 of 8 participants (25%)	1 of 11 participants (9%)
Low Oscillating Parasitemia	2 of 5 participants (40%)	3 of 10 participants (30%)	4 of 8 participants (50%)	5 of 11 participants (45.5%)

Through PCR it could be shown that one participant from Group B had a submicroscopic parasitemia throughout the entire time post-CHMI, although from a strain other than the one inoculated through the CHMI (NF54). Furthermore, there were four participants who had an infection through the CHMI-inoculated strain NF54, as well as an infection with a different strain. One of these mixed infection cases was with *P. malariae*, The other three were with a naturally acquired *P. falciparum* strain.

As soon as patients had malaria, defined as having both parasitemia and symptoms or a parasitemia of over 1,000, they were treated with artemether-lumefantrine (80mg/480mg) at the following timepoints: 0, 8, 24, 36, 48, and 60 hours, as the workflow chart depicts (see Figure 5). The time it took from CHMI until the development of malaria was similar between the four groups, as shown by the Kaplan-Meier plot in Figure 9. The four colored lines represent Groups A - D. As indicated by the cross found on the dark blue line on day 14, one data point was censored due to the one participant in Group B that was lost to follow-up due to a relocation to Cameroon. In order to compare whether the time until malaria occurred and thus treatment was provided for the groups is significantly different, a log rank test was performed. The p-value was 0.62, therefore there was no significant difference found in the onset of malaria between the four Groups.

A Forest plot was drawn for Cox proportional hazard models which can be seen in Figure 10. It compares Groups B, C, and D to Group A (the control group). At first sight, it seems that treatment B seems most risky (with a hazard ratio of 1.76), followed by D (with a hazard ratio of 1.24), then A (of course with a hazard ratio of 1, being the placebo), and C (with a hazard ratio of 0.64, so below 1). However, there is no significant difference between Groups B, C, and D and Group A, as the p-values of 0.489 for Group B, 0.654 for Group C, and 0.796 for

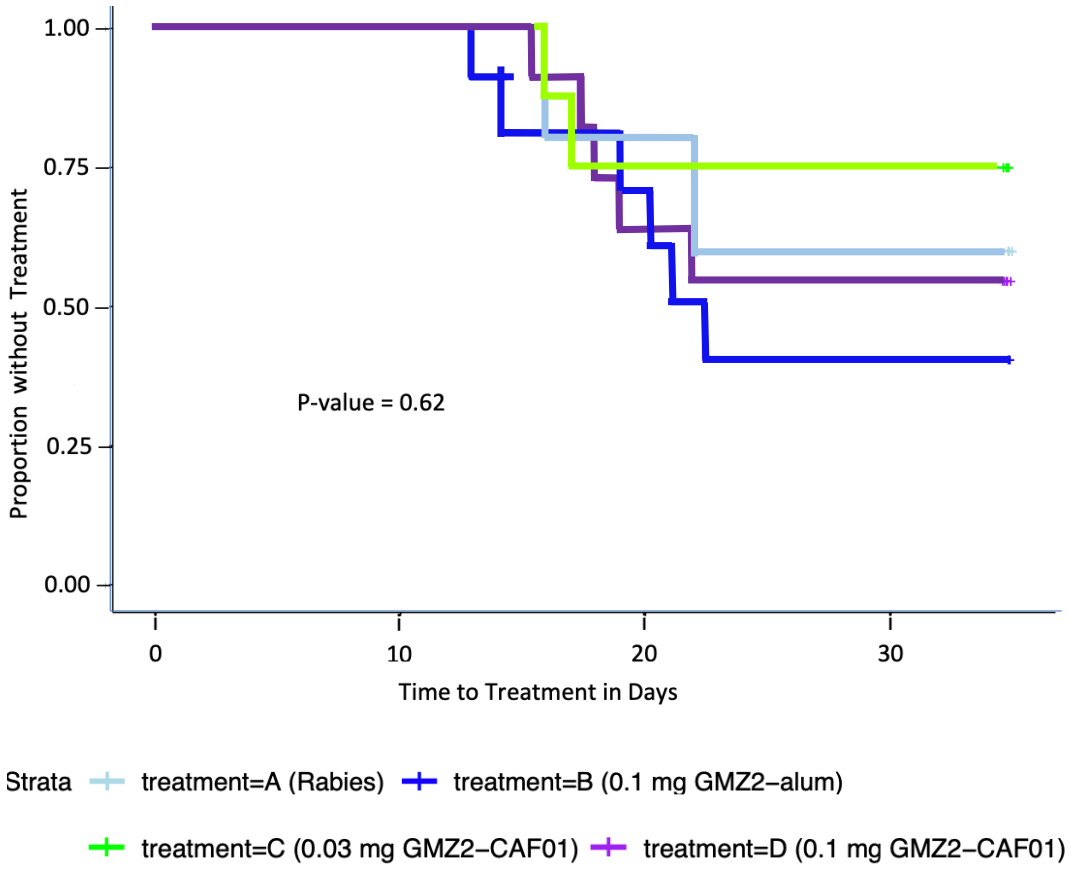

Figure 9: Kaplan-Meier Plot depicting the time from controlled human malaria infection (CHMI) to treatment of malaria, modified from [95]. Group A, who received rabies vaccine as a control, is shown in light blue. Group B, who received 0.1 mg GMZ2-Alhydrogel is shown in dark blue. Group C, who received 0.03 mg GMZ2-CAF01 is shown in green. Group D, who received 0.1 mg GMZ2-CAF01 is shown in purple. The log-rank test p-value is 0.62.

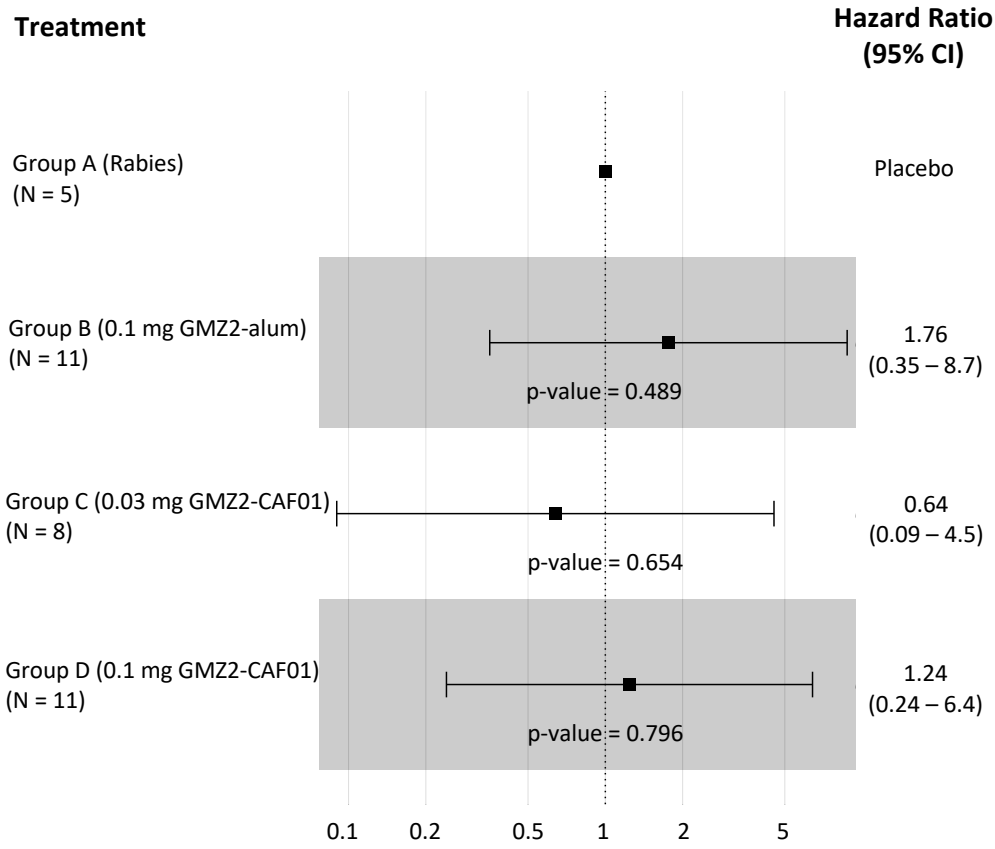

Figure 10: Forest plot for Cox proportional hazard ratios by the four treatment groups (A - D). The hazard ratios are indicated by the black squares and the values are furthermore shown on the right. The 95% confidence intervals are indicated by the horizontal lines, with the values furthermore shown in brackets below the hazard ratios.

Group D are all above 0.05. This can furthermotre be seen when looking at the large ranges of the 95% confidence intervals for the hazard ratios of Groups B, C and D. They all go from values below 1 to values above 1, e.g. for Group B the confidence interval is from 0.35 to 8.7, as depicted by the horizontal line, meaning the treatment could be better or worse than the placebo.

Figure 11 shows the development of individual parasitemia over the time of 35 days post CHMI. Note that if the line ends earlier, it was the case that the participant received malaria treatment before D35. The results are divided into the four groups that received CHMI, representing Groups A, B, C, and D. Parasitemia was measured through qPCR and depicted on a logarithmic scale as parasites per mL. The orange lines represent participants who developed malaria, the red lines represent participants who were protected from malaria, and the yellow lines represent participants who had low level parasitemia with no symptoms.

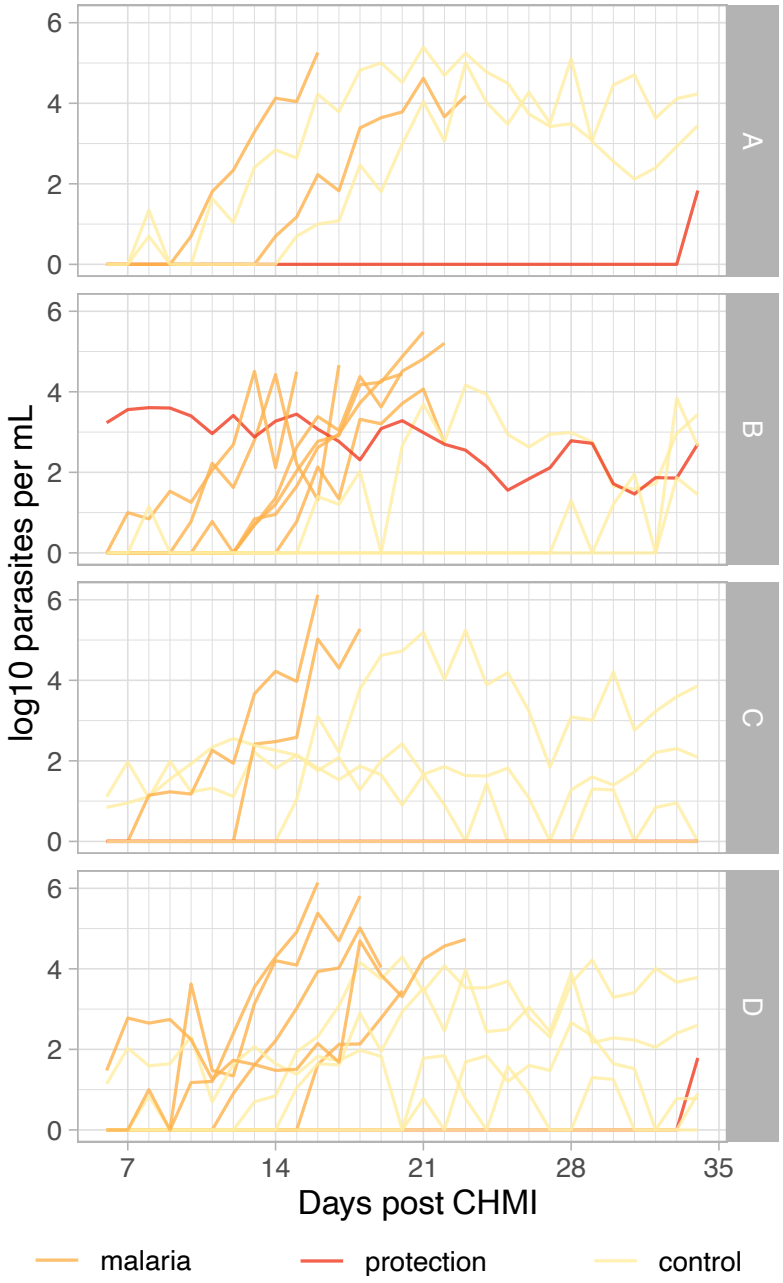

Figure 11: Development of participant parasitemia post CHMI (Controlled Human Malaria Infection) until day 35, measured by quantitative polymerase chain reaction, divided into Groups A-D (shown on the right). Lines end either on day 35, or earlier when malaria treatment had to be administered beforehand. Orange lines represent participants who developed malaria; red lines represent participants who were protected from malaria, and the yellow lines represent participants who had low level parasitemia with no symptoms. Modified from [95].

3.6 Summary of Results

In summary, the CHMI was well tolerated by the study participants. No SAEs occurred that could possibly be related to the study. Furthermore, the most common AE due to CHMI that was reported was headache as well as nausea.

The vaccine efficacy of the various formulations of candidate vaccines that were tested in this study turned out to not have a significant difference among each other. There was no significant difference observed regarding the efficacy between the two different adjuvants tested. Furthermore, there was no significant difference observed between the different dosages of the candidate vaccines tested. None of the interventions significantly changed the time to the development of malaria compared to the placebo treatment.

4 Discussion

Malaria remains a leading cause of death worldwide, especially among children in sub-Saharan Africa. Given the evolution of resistances against chemotherapeutics [97], as well as against insecticides [98], the development of a safe and effective malaria vaccine, ideally with a time- and cost-effective vaccination schedule, would be a milestone in the fight against this disease.

4.1 The Candidate Vaccine GMZ2CAF01

The candidate vaccine GMZ2 adjuvanted with CAF01 was shown to be safe and tolerable in this study, which was already published by Dejon-Agobe et al. [95]. As the idea of this vaccine was to mimic NAI, the goal was not to achieve sterile immunity, but rather to enable the body to withstand severe disease progression and the complications that follow from it. Furthermore, compared to pre-erythrocytic stage vaccines (see Section 1.7), blood-stage vaccine candidates have promising potential of not interfering with naturally acquiring immunity.

As already demonstrated in 1991, it is indeed possible to induce a protection through passive immunization, even when done with a different parasite strain than the one causing the infection, as Sabchareon et al. showed by transfusing IgG antibodies against *P. falciparum* from African adults into volunteers from Thailand [99]. These data lead to further research on the possibility of combining various vaccine candidates with different approaches in order to increase the immunity and decrease the severity of the disease, while also reducing parasite transmission rates. Certain difficulties do arise though when combining different vaccine types. These include possible interference with each other, and the effect of administering them in one formulation and at the same time and loca-

tion [100, 101]. However, promising results have also been published that do give hope in assuming a combination of various vaccines could be possible [102].

As it was shown that the efficacy of the vaccine correlated with increased vaccine-specific antibody titers [63], it would be desirable to find an adjuvant which promotes this increase. Unfortunately, contrary to what was expected, the adjuvant CAF01 did not perform in that regard, when compared to the adjuvant of Alhydrogel. However, this study was the first to compare CAF01 to another adjuvant directly, which can improve our understanding of how it fares. The results around the immunogenicity of GMZ2CAF01 in various dosages and compared to GMZ2 adjuvanted with Alhydrogel are discussed in the paper by Dejon-Agobe et al. [95]. It was shown that GMZ2 formulated with CAF01 did not result in higher immunogenicity compared to GMZ2 adjuvanted with Alhydrogel, which was proven through comparing the increase in vaccine-induced IgG levels of anti-GMZ2, anti-GLURP, and anti-MSP3 IgG [95].

Furthermore, a paper by Nouatin et al. performed on the same study as this thesis found that there was a correlation between the immunogenicity of the candidate vaccine GMZ2CAF01 and the presence of a helminth infection [103]. It would be helpful to have more research done on this correlation in order to better understand how helminth infections interfere with host immune responses, and therefore to be able to help understand what could be done to increase malaria vaccine efficacy.

4.2 Human Challenges in Africa

This study was the first where the method of CHMI with DVI of PfSPZ was used in order to assess the efficacy of a blood-stage candidate malaria vaccine in a

malaria endemic region. Moreover, it was one of the first times that CHMI was done on the African continent. While, as briefly mentioned in Section 1.8, CHMI studies have already been performed in Africa prior to this study, there is still little experience. The first was performed in 2012 in Bagamoyo, Tanzania, which is a malaria-endemic region, with the rationale of developing a model for using CHMI in an African setting [81]. The study enrolled 30 participants from higher learning institutions, of which 24 underwent CHMI and six were in a control group. The second CHMI in Africa was performed in 2013 in Nairobi, Kenya, which is a non-endemic region for malaria [83]. Again, the goal of the study was to develop the model of CHMI further, and their published findings were of great help to future CHMIs on the continent [84]. Following these, there was a study which was again performed in Tanzania between 2014 - 2015, with the aim to test the efficacy of a candidate malaria vaccine [104]. With 67 enrolled participants, of which 18 were in the control group, it was the largest CHMI trial in Africa. Meanwhile, between 2014 - 2016 in Lambaréné, Gabon (the same location that the here presented study was performed at), a CHMI was performed on 25 volunteers in order to develop the model further [82]. Following all these, the present study was performed.

Controlled human infections still face several challenges, of which one is the recruitment of participants. The here described trial did not have a bias towards higher-degree educated volunteers, such as for example the past CHMI in Kenya [83]. While this had the advantage of a higher likelihood of true informed consent, however, this was not a representative sample of the population, reducing the generalizability of the results. Another frequently discussed issue is the question on how to remunerate volunteers. There are arguments that justify a payment to compensate for costs incurred due to the participation in a study, such as travel costs, time absent from work, or childcare. Furthermore, there is a potential burden that the participant may have from participating, such as a potential infection

of a serious disease and the costs arising through circumstances such as isolation or absence from work. And lastly, although most controversial, it might be difficult to find volunteers that would participate without a financial incentive. This, however, might cause a recruitment bias of economically vulnerable populations. There has not yet been a final agreement in the research community around the topic of remunerating research participants, as described in detail in the book by Jamrozik et al. [72].

It is crucial to discuss all potential doubts and issues around controlled human infections extensively and openly, as it is very understandable that members of the public might have great concerns when they hear about this methodology. Some papers have already been published on the ethical implications and potential concerns [72–74, 105], but as long as there are still perceived risks in the general public, these must be addressed and the method modified accordingly, in order to make sure that these kinds of studies can continue to be performed, as they fast-track the paving of the road towards ending malaria-related death, and potentially also other diseases for which this method could be used in an ethical, justifiable manner.

When analyzing the results on the safety of the CHMI performed in this study, it can be seen that all the AEs that occurred that could at least possibly be related to the CHMI were all AEs that are typical for a malaria infection, as described in Section 1.3. Furthermore, there were no SAEs that occurred due to the performed CHMI. This makes CHMI through DVI comparable to the alternative way of infecting participants with malaria through CHMI by infected mosquitoes [76]. However, as the method through infected mosquitos gives an infection rate of 50% to 83%, as opposed to CHMI via DVI, which gives an infection rate of 100%, the method of CHMI through DVI is superior [77, 85, 106]. In fact, even the

alternative of waiting for a natural infection to occur through natural exposure gives comparable AEs, as the AEs, by definition, would be the same as for any naturally acquired malaria infection. As the traditional field trial method makes a study a lot more expensive and time consuming, and as also in the method of natural exposure the accuracy of being able to say with 100% likelihood that an infection occurred is not given, the CHMI through DVI outweighs all other options in multiple aspects.

4.3 Vaccine Trials on Semi-Immune Participants

The three studies that were performed on participants from endemic regions, as well as this study, were able to provide new insights into how semi-immune participants react to CHMI. Furthermore, this study underlines that efficacy testing of a candidate vaccine may give different results compared to studies performed on malaria-naive participants. A paper published on this same trial by Nouatin et al. was the first to find that the immunosuppressive molecule soluble Human Leukocyte Antigen (sHLA)-G might be a reason for this [107] . It seems that in semi-immune individuals, this molecule interferes with the vaccine efficacy [107]. Needless to say, it would be very prudent to do further research on this potential effect.

The here presented results showed that CHMI, when performed according to international safety and clinical trial standards, is a safe and excellent method for the measurement of efficacy of malaria treatments. For the field of malaria research, this is a unique success, as it allows for an immense acceleration of vaccine trials in the future, hopefully leading to an expedited progression to novel malaria vaccines, and ultimately to the elimination of this disease worldwide.

4.4 Limitations and Generalizability

4.4.1 Study Site and Study Design

Lambaréné in Gabon is a perfect site for CHMI and vaccination studies. The thorough experience that the team at CERMEL has gathered over the many years in which CERMEL has been operating, and the numerous clinical trials performed at this site, gave a solid foundation upon which to lean for this study. Multiple prior investigations that were performed in the region gave insights into baseline data on the environment and the population, minimizing the likelihood of any unforeseen events. Furthermore, a trained and experienced local team of researchers and field workers could provide support in all matters. If one were to replicate the entire study, or parts of it, such as CHMI, in a different study site, it would be vital to have a similar infrastructure and access to local know-how in place to ensure the safety of the participants.

Regarding the study design, it was proven to be very important to first clear any potential malaria infection through the treatment with clindamycin before the CHMI, as the study was performed in a malaria-endemic region, and it was therefore very likely that some of the participants were currently infected with malaria. However, even with this intervention, it was not possible to prevent a natural infection with malaria during the time from CHMI until C+35, as the people continued to live in the same, malaria-endemic region. In order to at least be able to differentiate such a natural infection from the infection through CHMI, continuous PCR testing was done in order to detect any potential natural infection by checking which genotype the malaria parasite had. Through this, a total of 5 natural infections could be confirmed (see Section 3.5.2). In order to prevent such a natural infection, one could have asked the volunteers to visit a region where

malaria is not endemic for the time of the study, but this would have been very costly and difficult for the volunteers to agree to with respect to their private and vocational responsibilities.

4.4.2 Study Population

A limitation of the study is that it solely included healthy male adults as participants. This restriction narrows the generalizability of the results obtained, as they might very well not be valid for women and children. This is a widespread phenomenon in research of treatments and vaccine efficacy studies [108]. The rationale given for excluding these demographics in scientific research varies, and, if any reason is given, it is mostly due to safety reasons, while sometimes there is no reason given at all. However, the detrimental consequences that this has had on science, drug and vaccine development and thus treatment and prophylaxis for female patients is not to be underestimated. This bias towards medical research having less accurate findings for women and children is ever-present, and concerns a group that is already often more vulnerable than adult men on many levels. Some research funders have been pushing to have scientist include women and minorities, but little has changed over the past years [109, 110]. Furthermore, it has also been shown that efficacy of vaccines in older people is significantly different compared to younger people [111], and again significant differences regarding efficacy of vaccines occur among older males versus females [112]. However, when looking at the other inclusion and exclusion criteria, they were in general more inclusive. For example, a BMI of less than 35 is relatively inclusive, although a study analyzing over 30,000 men and women from 13 African countries found that 23.3% of them were obese, defined as a BMI greater than 30 [113]. Lastly, as discussed in Section 4.2, a bias could be caused due to the methodology of how

participants are recruited for CHMIs.

Nevertheless, it must be noted that, by performing this study in Gabon rather than in a developed country, efforts were made to fight the general bias of not performing these kinds of studies in the countries where the treatment is most needed [114]. The good safety results obtained in this study are proof to the world that there should be less skepticism towards performing research studies on the African continent.

As it is children under the age of 5 that account for 80% of all malaria deaths on the African continent, the ultimate goal would be a malaria vaccine that would prevent these deaths [2]. Hence, the vaccine should be tested on this population set, and not on adult men only. However, as it is difficult to acquire informed consent from minors, and as this is a more vulnerable group, it makes sense to first test a candidate vaccine on adults that are more capable of articulating their needs and are capable of understanding their role and responsibilities. Once, after these studies would have provided good safety and efficacy data, one would move on to test the candidate vaccine on the target population.

The study explicitly called for adults that had lived their entire lives in a malaria-endemic region in order to guarantee the inclusion of participants who were semi-immune to malaria. However, this is not the target population for this candidate vaccine, as already mentioned in the preceding paragraph. The rationale behind the blood-stage candidate vaccine that was tested in this study is that it should help replicate such a semi-immunity to malaria, without having to be exposed to a potentially deadly malaria infection repeatedly during the course of a lifetime. Therefore the participants of this study were not the ideal target for this study. However, they were the best option for this clinical research stage.

4.5 Interpretations

Thus, although this study primarily aimed to assess the safety and efficacy of a candidate malaria vaccine, it is an equally interesting result of having done a CHMI in an endemic setting on the African continent. It sheds light on the potential that this method could have for future research on malaria interventions aiming to assess treatments or vaccines.

4.6 Conclusion

This study helped to show that CHMI is a safe and well-tolerated method, if performed by a well-trained, equipped, and experienced team. As there were no severe adverse events that arose during CHMI, it is possible to say that this method can be used for future studies on the African continent, provided certain standards and quality assurance are given on the ground.

Using CHMI we were able to assess the efficacy of the vaccine candidate GMZ2 in combination with the CAF01 adjuvant. It was shown that there was no significant difference in the development of clinical malaria after controlled infection between previous vaccination using GMZ2 adjuvanted with aluminum hydroxide and the novel adjuvant of CAF01. However, there was also no significant difference found in comparison to a control vaccination.

As an outlook, it would be interesting to study the effect of other adjuvants on GMZ2, as well as to combine blood-stage vaccines, such as GMZ2, with malaria vaccines that are active in the pre-erythrocytic stage. Regarding CHMI, it would be very beneficial for the rapid progression of malaria vaccine research to use this methodology more frequently. The way to malaria elimination is still a long one, and the international community will have to come up with different methods in order to expedite the fight against malaria.

5 Summary

5.1 Summary in English

Malaria is a life-threatening protozoan parasite disease transmitted by mosquitoes, which infected approximately 228 million people worldwide in 2018, of which 405,000 people died. Even with existing control methods, such as mosquito nets and insecticides, as well as various therapies, resistances to these methods are increasing as well. It would, therefore, be desirable if a vaccine against this disease were developed to tackle this problem sustainably. So far, there is only one vaccine that has been positively evaluated by scientists and is being tested in larger implementation studies in Africa, called RTS,S. Nevertheless, the effectiveness of this pre-erythrocytic vaccine is not yet satisfactory, which is why research continues to be carried out on various alternatives. One of these projects is the blood-stage vaccine candidate Recombinant *Lactococcus lactis* Hybrid GLURP and MSP3 (GMZ2), which was tested in this study. Previous studies in both animals and humans showed that this candidate proved to be well-tolerated and produced a convincing antibody profile. Since these studies used aluminum hydroxide as the adjuvant, a further boost was expected by using the novel Cationic Adjuvant Formulation 01 (CAF01), which could further increase the immunity and, ultimately, the effectiveness. GMZ2CAF01 tries to induce the semi-immunity that occurs in people who permanently live in endemic malaria regions, thereby controlling the multiplication of the pathogen in the blood. Based on a Controlled Human Malaria Infection (CHMI), this study tried to test the efficacy of the vaccine candidate GMZ2CAF01, as well as to make a statement about the safety of the methodology of the CHMI. 50 healthy Gabonese male participants with lifelong exposure to malaria were randomly placed into five groups: Group A received

a rabies vaccine as placebo, Group B received 100 μg GMZ2-Alhydrogel, Group C received 30 μg GMZ2-CAF01, and Group D and E received 100 μg GMZ2-CAF01. All but Group E received subsequent CHMI via direct venous inoculation (DVI) with 3,200 *P. falciparum* sporozoites (PfSPZ). This methodology was previously developed at the Institute of Tropical Medicine, University of Tübingen, to guarantee a 100% infection with malaria. Subsequently, the subjects were questioned and observed for any adverse event (AE)s of the CHMI, and regular blood tests were carried out to determine parasitemia through microscopy, which was confirmed by means of Polymerase Chain Reaction (PCR). As soon as either a parasitemia of over 1,000 or a lower parasitemia accompanied by malaria symptoms was detected, the volunteers were treated with artemether-lumefantrine. The remaining participants were treated with artemether-lumefantrine after day 35 post CHMI. It could be confirmed that CHMI proved to be safe and that there were no serious adverse events (SAE)s that occurred. However, almost all subjects experienced at least one AE, of which Grade 1 headache was the most common AE. There was no significant difference between the groups in terms of both the occurrence of malaria and the time until malaria occurred. In conclusion, it can be said that the GMZ2CAF01 vaccine candidate did not induce the semi-immunity that was desired. However, CHMI proved to be a safe and promising method for studying malaria immunization and therapies.

5.2 Zusammenfassung

Malaria ist eine durch Stechmücken übertragene lebensbedrohliche parasitäre Infektionskrankheit. Im Jahr 2018 waren weltweit etwa 228 Millionen Menschen betroffen, von denen etwa 405.000 Menschen starben. Auch bei den aktuell verfügbaren Bekämpfungsmethoden wie Moskitonetze, Insektizide sowie verschiedene medikamentöse Therapien nehmen Resistenzen bei diesen Kontrollmaßnahmen zu. Es wäre daher wünschenswert, wenn ein Impfstoff gegen Malaria entwickelt werden könnte, um dieses Problem nachhaltig anzugehen. Bisher gibt es nur den Impfstoff RTS,S, der positiv evaluiert wurde und in größeren Implementierungsstudien in Afrika getestet wurde und inzwischen entsprechend den Empfehlungen der WHO eingesetzt wird. Dennoch ist die Wirksamkeit dieses präerythrozytären Impfstoffs noch nicht zufriedenstellend, weshalb weiterhin an verschiedenen Alternativen geforscht wird. Eines dieser Projekte ist der Blutstadium-Impfstoffkandidat Recombinant *Lactococcus lactis* Hybrid GLURP and MSP3 (GMZ2), der in der vorliegenden Studie getestet wurde. Frühere Studien an Tieren und Menschen zeigten, dass sich dieser Kandidat als gut verträglich erwies und ein überzeugendes Antikörperprofil induzierte. Da in den bisherigen Studien Aluminiumhydroxid als Adjuvans verwendet wurde, wurde durch die Verwendung der neuartigen Substanz Cationic Adjuvant Formulastion 01 (CAF01) ein weiterer Schub erwartet, der die Immunität und letztlich die Wirksamkeit weiter steigern könnte. Mit GMZ2CAF01 wird versucht, bei Menschen, die dauerhaft in Malaria-Endemiegebieten leben, Semiimmunität zu induzieren und so die Vermehrung des Erregers im Blut zu kontrollieren. Basierend auf einer kontrollierten Malariainfektion (Controlled Human Malaria Infection (CHMI)) versuchte diese Studie, die Wirksamkeit des Impfstoffkandidaten GMZ2CAF01 zu untersuchen und eine Aussage über die Sicherheit der Methodik der CHMI zu treffen. 50 gesunde gabunische männliche Teil-

nehmer mit lebenslanger Malaria-Exposition wurden nach dem Zufallsprinzip in fünf Gruppen eingeteilt: Gruppe A erhielt einen Tollwutimpfstoff als Placebo, Gruppe B erhielt 100 μg GMZ2-Alhydrogel, Gruppe C erhielt 30 μg GMZ2-CAF01 und Gruppe D und E erhielten 100 μg GMZ2-CAF01. Alle außer Gruppe E erhielten anschließend CHMI über direkte venöse Inokulation (DVI) von 3.200 *Plasmodium falciparum*-Sporozoiten (PfSPZ). Diese Methodik wurde zuvor am Institut für Tropenmedizin der Universität Tübingen entwickelt, um eine 100-prozentige Infektion mit Malaria zu garantieren. Anschließend wurden die Probanden befragt und bzgl. unerwünschter Ereignisse (AE) der CHMI beobachtet, und es wurden regelmäßige Bluttests durchgeführt, um Parasitämien mikroskopisch zu bestimmen, was dann mittels Polymerase-Kettenreaktion (PCR) bestätigt wurde. Sobald entweder eine Parasitämie von über 1.000 / μl Blut oder eine niedrigere Parasitämie mit Malariasymptomen festgestellt wurde, wurden die Probanden mit Artemether-Lumefantrin behandelt. Die restlichen Teilnehmer wurden nach Tag 35 nach der CHMI mit Artemether-Lumefantrin behandelt. Es konnte bestätigt werden, dass sich CHMI als sicher erwies und dass keine schwerwiegenden unerwünschten Ereignisse (SAE) auftraten. Bei fast allen Probanden kam es jedoch zu mindestens einem AE, wobei Kopfschmerz 1. Grades das häufigste AE war. Es gab keinen signifikanten Unterschied zwischen den Gruppen sowohl hinsichtlich des Auftretens von Malaria als auch der Zeit bis zum Auftreten von Malaria. Zusammenfassend kann gesagt werden, dass der Impfstoffkandidat GMZ2CAF01 nicht die gewünschte Semiimmunität induzieren konnte. Es hat sich jedoch deutlich gezeigt, dass CHMI eine sichere und vielversprechende Methode zur Untersuchung von Malaria-Immunisierung und -Therapien ist.

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7 Personal Contribution to this Study

The study was designed by Professor Dr. Benjamin Mordmüller and coordinated by Dr. Ayola Akim Adegnika. The principal investigator was Dr. Ulysse Ateba Ngoa. The clinical trial investigation team was subdivided into a clinical investigation sub-team led by Dr. Jean Claude Dejon-Agobe, a field work sub-team led by Mr. Olivier Koumba, an immunology investigation sub-team led by Dr. Odilon Nouatin and a Malaria Diagnostic sub-team led by Julie Engelhorn. The role of the Malaria Diagnostic sub-team was critical for this study with regard to patient safety and assessment of vaccine efficacy. Indeed malaria diagnostic was essential for ensuring patient safety as the patients were inoculated with a live strain of Plasmodium falciparum that could cause malaria. Moreover, vaccine efficacy was evaluated based on the result of the malaria diagnostic.

As the lead of the Malaria Diagnostic sub-team, Julie Engelhorn supervised a team of 11 laboratory technicians. In her role she designed and implemented completely new and highly efficient processes that allowed on-time collection of patient blood samples, processing of these samples and reporting of results to the clinical investigation sub-team. While supervising the following steps, she was herself involved in collecting the samples, preparing the thick blood smear (TBS) and performing manual detection of Plasmodium falciparum using a microscope to read the TBS. As the lead of the Malaria Diagnostic sub-team she maintained quality results by participating in the laboratory quality assurance program; proposed and made adjustments in procedures; reported results according to protocols; generated reports; maintained records.

In addition, Julie Engelhorn actively participated in meetings with the clinical investigation sub-team and regularly liaised with the team members for the evaluation of adverse events that could be related to malaria. She advised and provided

suggestions on steps to take for proper malaria diagnostic. She resolved problems presented by the clinical investigation sub-team by consulting with other laboratory managers, technical coordinators, and laboratory directors.

8 Erklärung zum Eigenanteil der Dissertationsschrift

Die Arbeit wurde in der Medizinischen Universitätsklinik und Poliklinik Tübingen, Abteilung VII – Tropenmedizin (Schwerpunkt: Institut für Tropenmedizin, Reisemedizin, Humanparasitologie) und dem Centre de Recherches Médicales de Lambaréné (CERMEL) unter Betreuung von Herrn Professor Dr. Peter Kremsner durchgeführt.

Die Konzeption der Studie erfolgte durch Prof. Dr. med. Benjamin Mordmüller, Dr. Ulysse Ateba Ngoa, und Prof. Dr. med. Ayola Akim Adegnika. Sämtliche Arbeit um die Malaria Diagnostik wurde von mir eigenständig durchgeführt, assistiert von einem Team von 11 Mitarbeitenden. Die Befragungen der Studienteilnehmer für die Datenerhebung bezüglich Sicherheit wurden durchgeführt durch Dr. Ulysse Ateba Ngoa, Dr. Jean Ronald Edoa, Dr. Jean Claude Dejon-Agobe und Andreas Homoet. Die statistische Auswertung erfolgte eigenständig durch mich. Ich versichere, das Manuskript selbständig verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben. Die Publikation wurde von allen Autoren unter der Supervision von Prof. Dr. med. Benjamin Mordmüller geschrieben.

9 Publication

Jean Claude Dejon-Agobe, Ulysse Ateba-Ngoa, Albert Lalremruata, Andreas Homoet, Julie Engelhorn, Odilon Paterne Nouatin, Jean Ronald Edoa, José F. Fernandes, Meral Esen, Yoanne Darelle Mouwenda, Eunice M. Betouke Ongwe, Marguerite Massinga-Loembe, Stephen L. Hoffman, B. Kim Lee Sim, Michael Theisen, Peter G. Kremsner, Ayôla A. Adegnika, Bertrand Lell, and Benjamin Mordmüller

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