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Safety and immunogenicity of GMZ2 adjuvanted with aluminum hydroxide and CAF01 in semi-immune Gabonese volunteers and the impact of helminth infection on vaccine immunogenicity

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Homoet, Andreas Bernhard Maria

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Dekan:	Professor Dr. B. Pichler
1. Berichterstatter:	Professor Dr. B. Mordmüller
2. Berichterstatter:	Professor S. Wagner, PhD
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Dedication

Dedicated to my family.

„Ich bin Leben, das leben will, inmitten von Leben, das leben will.“
Albert Schweizer

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A. lumbricoides	*Ascaris lumbricoides*
ACT	Artemisinin combination therapy
ADCI	Antibody dependent cellular inhibition
AE	Adverse event
ALT	Alanine transaminase
AMA-1	Apical membrane antigen 1
APC	Antigen presenting cell
AST	Aspartate transaminase
BCG	Bacillus Galmette-Guerin
BMI	Body mass index
BSV	Blood-stage vaccine
CAF01	Cationic adjuvant formulation 01
CHMI	Controlled human malaria infection
DDA	Dimethyldioctadecylammonium
DDT	Dichlorodiphenyltrichloroethane
ELISA	Enzyme-linked immunosorbent assay
GCP	Good Clinical Practice
GLURP	Glutamate rich protein
GMEP	Global Malaria Eradication Program
HBs	Hepatitis B surface antigen
HGB	Hemoglobin
HIV	Human immunodeficiency virus
IFN-γ	Interferon gamma
IgG	Immunoglobulin Gamma

IL	Interleukin
i.m.	Intramuscular
iRBC	Infected red blood cell
IRS	Indoor residual spraying
ITN	Insecticide treated bed net
LDH	Lactate dehydrogenase
Leu	Leucocytes
L. loa	*Loa loa*
Lym	Lymphocyte
MSP3	Merozoite surface protein 3
NAI	Natural acquired semi-immunity
Neu	Neutrophil
P. falciparum	*Plasmodium falciparum*
P. ovale	*Plasmodium ovale*
P. vivax	*Plasmodium vivax*
P. knowlesi	*Plasmodium knowlesi*
PAM	Pregnancy-associated malaria
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PfEMP1	*Plasmodium falciparum* erythrocyte membrane protein 1
PfSPZ	*Plasmodium falciparum* sporozoite
Plt	Platelets
p.d.	Per dose
PRR	Pattern recognition receptor
RBC	Red blood cell

RDT	Rapid diagnostic test
RESA	ring-infected erythrocyte surface antigen
S. haematobium	*Schistosoma haematobium*
TBC	Tuberculosis
TBS	Thick blood smear
TBV	Transmission blocking vaccine
TDB	α,α'-trehalose 6,6'- dibehenate
T_H	T helper
T_{reg}	Regulatory T cells
T. trichiura	*Trichuris trichiura*
WBC	White blood cell
WHO	World Health Organization

1. Introduction

1.1 Malaria: A persistent public health concern

Malaria is a vector-borne disease caused by intracellular protozoan parasites of the *Plasmodium* (*P.*) genus. The disease had accompanied mankind even before human ancestors diverged from the great apes[1]. Arising from its estimated origin, the Ethiopian areas, it followed the migration of people spreading over most of the populated parts of the world including Asia, North America and Europe.

Due to global efforts within the Global Malaria Eradication Program (GMEP) of the World health Organization (WHO), which was mainly operating with insecticides such as dichlorodiphenyltrichloroethane (DDT) and anti-malarial drugs (e.g. chloroquine and sulfadoxine-pyrimethamine), malaria was eliminated from North America, Europe, parts of Asia and South America in the mid to late-twentieth century[2]. Despite the success in temperate climate zones, no major success could be achieved in the sub-Saharan region of Africa. Moreover, the program struggled with the economic crisis in 1970s and the subsequent cutting of funding for malaria control as well as the occurrence of first drug resistances and the restriction of DDT due to environmental and health hazards. As a result, the GMEP failed and malaria reappeared in many areas, and in the consistently malaria endemic Sub-Saharan region the death rate increased over the factor 1.5[1,3].

In the early 21st century malaria was re-recognized as a serious global health issue and the Roll Back Malaria initiative was created. Since then eight countries have eliminated malaria, the global incidence and mortality have declined by 37% and 60%, respectively. For children under five, malaria death rates have declined by 43% worldwide[4,5]. This success was based on three key interventions: Insecticide-treated bed nets (ITN), indoor residual spraying (IRS) and artemisinin combination therapies (ACT). Further strategies comprised rapid diagnostics tests (RDT), efficient drug surveillance, strong public health communications and behavioral change programs[6].

Nevertheless, despite global endeavors, further progress has come to a halt and the number of cases per 1000 population at risk has stood at 59 for the past three years[7]. Furthermore, previous achievements are fragile as proven by the outbreak of the Ebola

virus in West Africa in 2014 and its devastating impact on the basic health service and thus on malaria control[8].

The current efforts are not sufficient to eradicate malaria and their efficacy is threatened to be impaired: ITN and IRS rely on a few insecticide classes particularly pyrethroids and emerging insecticides resistance has already covered nearly two thirds of countries with ongoing malaria transmission[9]. Further, the third key intervention, highly effective artemisinin-based therapies, is threatened by emerging anti-malarial drug resistance as further outlined in the malaria treatment section[10].

Therefore, substantial challenges in the fight against malaria remain. In 2017, there were still an estimated 219 million cases of malaria and 435,000 deaths worldwide[7].

1.1.1 Malaria distribution

Malaria occurs in countries located within a belt around the tropical and subtropical latitudes (figure 1)[11]. Most of these cases occurred in the WHO African Region (200 million or 92%). Five countries account for nearly half of all malaria cases worldwide: Nigeria (25%), Democratic Republic of the Congo (11%), Mozambique (5%), India (4%) and Uganda (4%)[7].

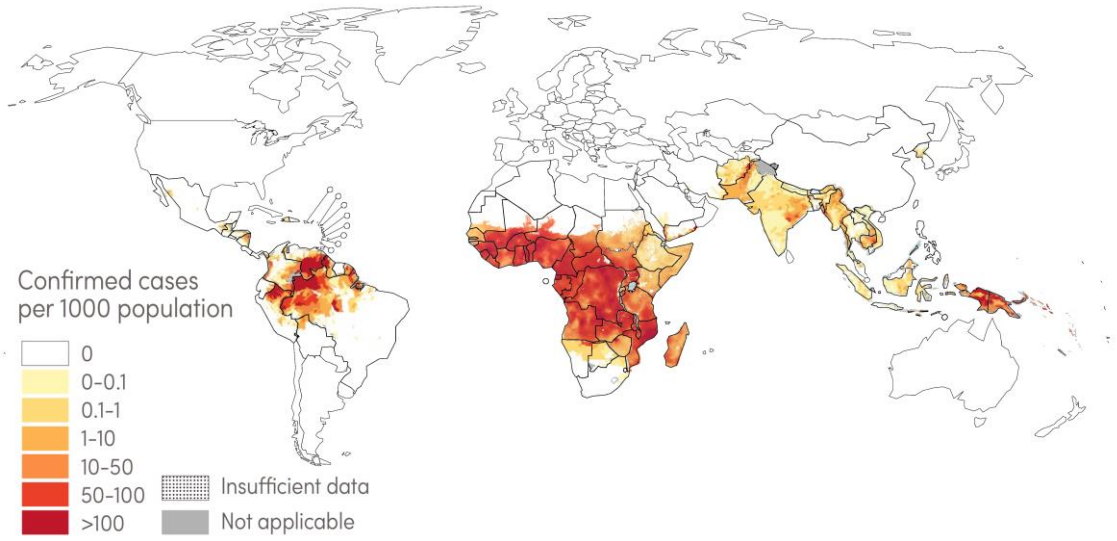


Figure 1: *Countries with ongoing malaria transmission 2017, freely adapted from the country reports of the WHO Malaria Report 2018* [7].

The majority of deaths concern children less than 5 years of age (78% of all deaths). Other vulnerable populations are malaria-naïve migrants, mobile populations, travelers and subjects with impaired immune system such as people infected with the human immunodeficiency virus (HIV), or pregnant women. Especially primigravida almost triples the risk of severe malaria[5,12].

The concentration of malaria cases in sub-Saharan Africa can be related to several factors such as meteorological condition (e.g.: precipitation, humidity and temperature), as well as local disease control capacities, and vector breeding preferences[13].

1.1.2 Anopheles vectors

Malaria is a vector-borne disease transmitted by bites of feeding female A*nopheles* mosquitos. There are 512 *Anopheles* species recognized worldwide from which 70 are able to transmit *Plasmodium spp.* to humans[14]. While all *Anopheles* mosquitoes breed in water, each species has its particular breeding preferences. They differ from shallow transient collections of fresh water (hoof prints, irrigation ditches or puddles) to permanent bodies of water (swamps, or lagoons). Female *Anopheles* mosquitos need blood as a protein source for egg development. Depending on the ambient temperature, their eggs develop within 5-14 days into matured mosquitos. Being crepuscular or nocturnal, they are mainly active from dusk till dawn feeding on nectar, fruits, and other sources of sugar.

Among the 512 Anopheles species 41 are defined as "Dominant Vector Species" (DVS). DVS are recognized as the most important malaria vectors. The Anopheles Gambiae complex found in Africa represent the most effective and efficient DVS. There are 4 principal species belonging to *An. gambiae complex: An. gambiae, An. arabiensis, An. merus* and *An. melas*.

1.1.3 *Plasmodium* species

The malaria parasite is an eukaryotic, apicomplexan, unicellular *protozoan* of the *Plasmodium* genus. There are five different species known to affect humans: *Plasmodium falciparum (P. falciparum), Plasmodium ovale (P. ovale), Plasmodium vivax (P. vivax), Plasmodium malariae (P. malariae), and Plasmodium knowlesi (P. knowlesi)*[15].

P. falciparum

P. falciparum is the most prevalent malaria parasite in the WHO African Region (accounting for 99.7% of estimated malaria cases in 2017) as well as in the WHO regions of South-East Asia (62.8%), the Eastern Mediterranean (69%) and the Western Pacific (71.9%)[7]. *P. falciparum* is the focus of this thesis as it is targeted by the GMZ2 vaccine candidate. *P. falciparum* causes the most dangerous form of malaria known as malaria tropica. Infection with *P. falciparum* affects red blood cells (RBC) across all ages and without treatment it leads to the heaviest parasites burden. Moreover, due to the adherence to microvascular endothelial cells, *P. falciparum* can disrupt the microvascular blood flow and induces endothelial dysfunction. This leads, along with other factors, to impaired tissue perfusion[16,17]. *P. falciparum* is the main cause of severe malaria.

P. vivax and *P. ovale*

P. vivax and *P. ovale* are the cause of the malaria tertian, which is named after the characteristic fever episodes occurring every 48 hours. As result of a dormant liver stage called hypnozoite, they can relapse after months or years. *P. ovale* has typically lower parasitemia compared to the other *Plasmodium species.* It is mainly found in sub-Saharan Africa and the islands of the western pacific[18]. *P. ovale* consists of two species (*P. ovale curtisi* and *P. ovale wallikeri*)[19]. *P. vivax* has a broader distribution due to sporogonic development at lower temperatures. However, it is limited in Africa due to the absence of the required duffy receptor on RBC in many African populations. Despite the historical name "benign tertian malaria", *P. vivax* infection has a substantial burden of disease. Even if it is less virulent compared to *P. falciparum,* it induces similar anemia, which can lead severe illness and fatal outcomes particularly when associated with co-morbidities[20,21].

P. malariae

P. malariae induces malaria quartana with fever episodes occurring every 72 hours. Its distribution coincides at large with *P. falciparum.* The prepatent period is wide ranged from 16 to 59 days and extended compared to the other plasmodia (6-27 days). The prolonged fever cycle, targeting of elder erythrocytes in the blood-stage and the lower amount of merozoites per infected red blood cell (iRBC) leads to comparative lower maximum blood parasites densities. *P. malariae* does not relapse from dormant liver stages, nevertheless it can persist for an extensive period in the blood at very low densities and recrudesce after decades. Moreover, malaria quartana can cause the nephrotic syndrome[22].

P. knowlesi

P. knowlesi is mainly found in Southeast Asia. It was first described in infected macaques in 1931 and not till 1965 reported as a naturally acquired human infection due to difficulties distinguishing it from other plasmodia by microscope. By now, there is no evidence of man-to-man transmissions and therefore the disease is considered zoonotic. Nevertheless, *P. knowlesi* poses a threat in forested areas in Southeast Asia[23].

1.1.4 Life cycle and pathophysiology

The life cycle of the *Plasmodium* parasite is complex and features altering intra and extra cellular life stages in both vertebrate and arthropod hosts (figure 2).

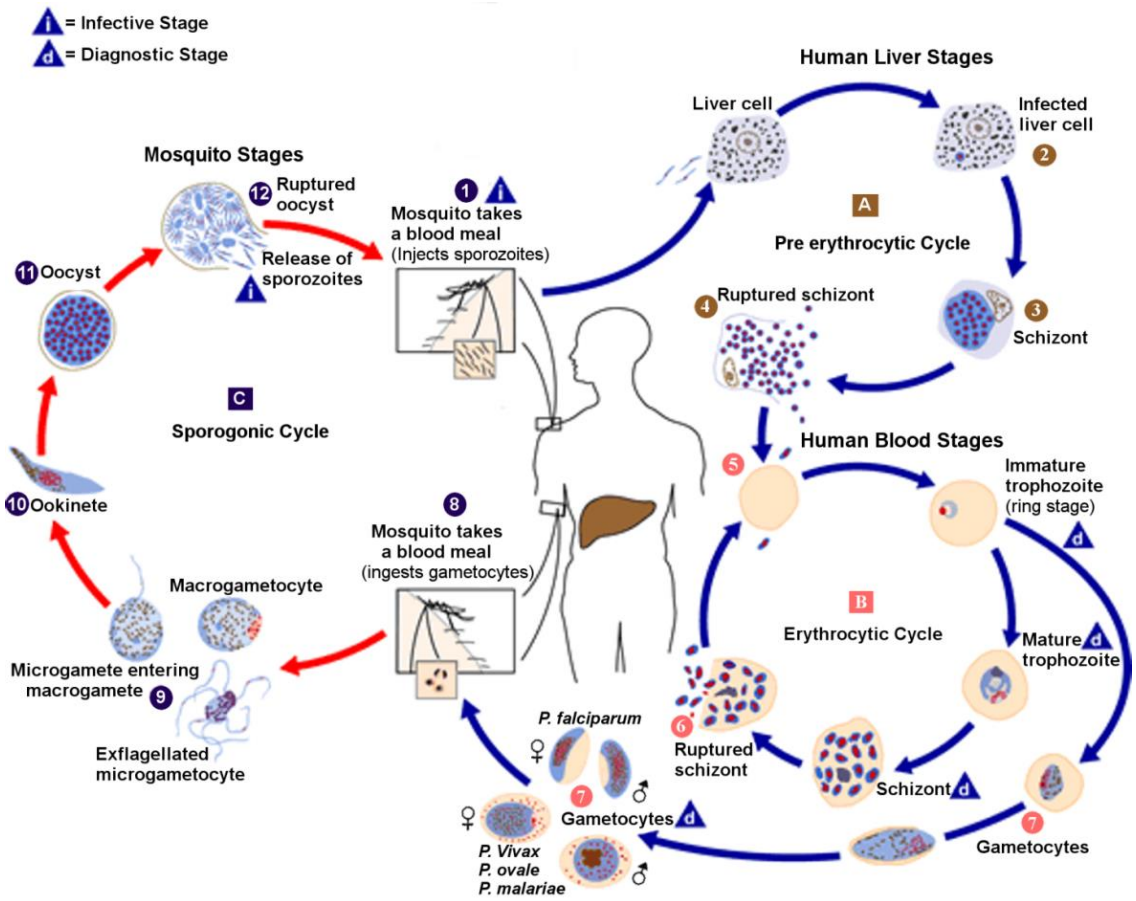


Figure 2: *Life cycle of plasmodia, Center of Disease Control (CDC)* [24]*.While feeding on the human host, the infected mosquito inoculates motile sporozoites. They actively navigate through the skin in pursuit of a capillary vessel to be flushed into the liver [1]. After 15-45 min, they invade hepatocytes [2], remain there for 9-16 days maturing into schizonts [3] and undergo exo-erythrocytic schizogony (A). During this stage, P. vivax and P. ovale can develop dormant liver stages (hypnozoite). With the rupture of the liver cell 2000-30,000 merozoites are released [4], which actively infect RBCs [5] and initiate the erythrocytic schizogony (B). The now called trophozoites develop into schizonts, which releases 8-32 merozoites by rupture [6], which continue the cycle by re-infecting further RBCs. This leads to an approximately ten-fold increase of iRBC every 48 hours. The feed-forward loop with exponential multiplication continues until limited by metabolic resource-restriction, the immune system, or anti-malarial treatment. The parasitemia can built up to* 10^{13} *parasites, which causes the clinical symptoms*[25]*. A small fraction of the merozoites develop into longer living sexual forms (gametocytes) [7], which are ingested*

by a blood meal taking anopheles mosquito [8]. The sexual proliferation in the mosquito is called sporogonic cycle (C). In the gut of the mosquito the gametocytes are released from the erythrocytes. The microgametes (male form) fertilizes the macrogametes (female form) forming zygotes [9], which develop into motile ookinetes and encysts in the midgut wall of the mosquito [10, 11]. By mitosis and meiosis thousands of haploid sporozoites are created and released during the rupture of the mature oocyst [12]. After migrating to the salivary glands, the sporozoites finish their differentiation and await inoculation into the next human host during a subsequent mosquito bite [1].

1.1.5 Immune response to *P. falciparum*

Plasmodium spp. are immune-evasive parasites exerting high antigenic variation throughout their life cycle[26,27]. The response of the human immune system is an equally complex interplay of the native and adaptive system as shown in figure 3. It consists of humoral and cellular immune response including antibodies, cytokines, regulatory and effector T cells, neutrophil and monocyte activation[28]. However, the specific mechanisms mediating these immune cascades remain poorly understood[29] and sterile immunity is typically not achieved[30].

Thousands of years of co-evolution between the parasite and the human host have induced an immense evolutionary pressure exerted by the human immune system. This has selected for an extensive polymorphism of parasite genes encoding immunodominant antigens, known as antigenic variation[31–33], whereas the functionally relevant and conserved protein regions of the genes are often poorly immunogenic, which subsequent leads to immune evasion[34]. Most antigens of the sporozoite, liver and sexual blood-stage are rather conserved, compared to antigens of merozoite and surface of iRBCs, which are extremely polymorphic[31]. Furthermore, evidence suggests that parasites can also effectively modulate the host immune system[35,36]. This permits reinfections and the potential chronic character of the disease[30,32]. A fragile equilibrium between pro-inflammatory and regulatory responses affects the outcome of an infection[37] and has been proposed as target for immune-modulatory interventions[38].

Naturally acquired immunity (NAI) can develop following repeated infections. It is robust but usually not sterile and titled semi-immunity in the malaria field. It requires recurrent infections over a prolonged period, depends on the degree of exposure, and can be further subdivided into: (I) A rather rapidly acquired anti-disease immunity which

impairs the chance of severe clinical outcome within a same level of parasitemia; (II) A relatively slower developed anti-parasite immunity, which diminishes parasite densities and therefore alleviates the clinical symptoms.

One possible mechanistic model for the development of NAI is called premunition, where chronic low grade asymptomatic parasitemia is proposed to protect from severe clinical outcomes of new infections[30,39]. However, one remains susceptible for malaria infections and if the reinfection rate falls below a certain threshold, the protection wanes over time. The anti-disease immunity has an estimated half-live of 5 years whereas the slower acquired antiparasitic immunity has an estimated half-life of 20 years[30].

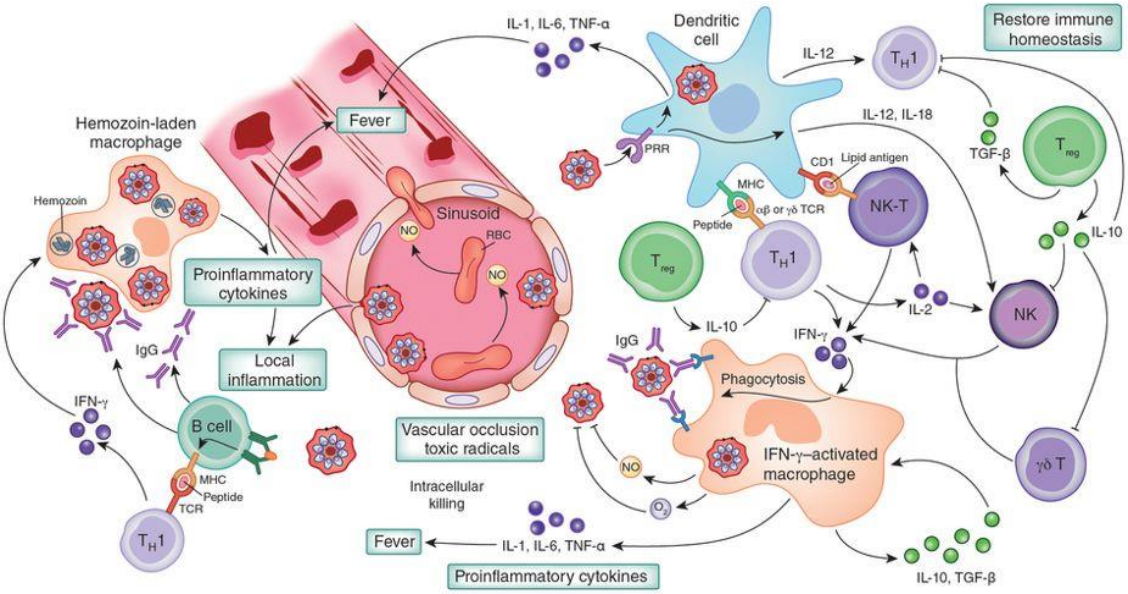
Humoral immunity

Antibodies are related to protection as several passive transfer studies in humans have shown since 1960[40–42]. The humoral immune response to malaria is mainly characterized by immune-globulin (Ig)-G, whereas the roles of the other immunoglobulin classes like IgM, IgA and IgE are not well described[43]. Antibodies can be effective (e.g. inhibiting), neutral or contra productive due to blocking of effective antibodies. In the latter case, they can even have a negative effect[44]. The protection of IgG is mainly mediated by the cytophilic subclasses IgG1 and IgG3[45]. At the pre-erythrocytic life stage antibodies can immobilize and opsonize the inoculated sporozoites until they invade hepatocytes[46]. During the intracellular hepatic stage, the parasite is not targetable by antibodies. Throughout the subsequent erythrocytic stage merozoites-specific antibodies are likely to play an important role. They agglutinate and opsonize the parasite, which leads in cooperation with effector cells to phagocytosis, complement mediated damage[44], prevention of further RBC invasion[47] and Antibody-Dependent-Cellular-Inhibition (ADCI). This is further boosted by lymphocytes, which produce interferon γ (IFN-γ) and subsequently activate macrophages. Further important antibodies target antigens of infected RBCs and inhibit their sequestration in capillary vessels.

Cellular immunity

Once the sporozoites reach the liver and invade hepatocytes, they are concealed against antibody targeting. Hence, the now intracellular parasite is targeted by the cellular part of the immune system. The major role play $CD8^+$ T cells, which start immune cascades including $CD4^+$ T cells, natural killer cells, interleukin(IL)-12, IFN-y and NO as final

effector[44]. Moreover, $CD4^+$ T cells can have a major impact on the nature of the over-all immune response depending on their predominant subset. Subsets are characterized by their distinct chemokine secretion profiles and their different expression of surface receptors[48]. Balance between inflammatory and non-inflammatory immune responses appears to be key for the clinical outcome of a malaria episode[49]. A strong early T_H1-mediated pro-inflammatory answer is needed to reduce parasite growth and facilitate the clearance of iRBCs, whereas an unregulated excessive response may lead to immunopathology and severe malaria[31].



Riley and Stewart 2013, Figure 2

Figure 3: *Immune response against malaria. Parasites or iRBCs are recognized by dendritic cells (DC) through pattern recognition receptors (PRRs). This leads to phagocytosis and subsequent presentation of pathogen-antigens to T cells. Depending on the signaling of the PRR, cytokines are secreted, which direct the T cell differentiation, cause the inflammation and malaria pathogenesis. T_H1 cells support B cell maturation and antibody production. Moreover, they activate macrophages through IFN-γ secretion. Activated macrophages in turn phagocytose opsonized parasites and release proinflammatory cytokines. Endothelial cells express adhesion molecules, which bind infected RBC. The restoration of immune homeostasis is induced by anti-inflammatory cytokines secreted by regulatory T cells (T_{reg}) and makrophages[31]. Reprinted with permission from Nature Publishing Group.*

1.1.6 Clinical appearance

Uncomplicated malaria

After a clinically silent incubation period of approximately 14 days (up to 4 weeks for *P. falciparum*) *P. falciparum* infection can result in a range of symptoms going from minor to severe and potentially fatal. A typical sign of malaria is the appearance of fever. Its frequency is related to the erythrocytic schizogony. During the synchronized rupture of infected RBC, accumulated parasite products like hemozoin are released into the bloodstream. This induces an immune response triggering fever, rigor and other inflammatory responses related to malaria. Characteristically this ague occurs every 48 hours with *malaria tertiana* and every 72 hours with malaria quartana. Malaria tropica however demonstrates irregular fever attacks due to less synchronized erythrocytic schizogony. Even though a three-stage progression of disease (cold, hot and sweating stage) is called classic, it is rarely observed. More common is a combination of symptoms such as: Fever, chills, fatigue, headaches, perspiration, myalgia, nausea, vomiting, cough general malaise and abdominal discomfort. Physical examination may reveal an enlarged spleen, mild jaundice as well as an enlargement of the liver. Characteristic but not obligatory is a decreased platelet (plt.) count, elevated total bilirubin and lactate dehydrogenase (LDH) [17,50,51]. Additional findings may occur depending on the progress of the disease and the extend of organ dysfunctions. If not treated properly at this stage, the parasite burden may increase and complications occur within days or even hours depending on the virulence of the pathogen and the susceptibility of the host[17,50,52,53].

Severe malaria

Even though infections with *P. vivax* and *knowlesi* can result in severe malaria, *P. falciparum* is responsible of the vast majority of severe malaria cases[51]. Severe malaria is defined by clinical or laboratory evidence of vital organ dysfunction in the absence of an identified alternative cause, and in the presence of *P. falciparum* asexual parasitaemia[51,52,54]. Several definitions are used for the classification of severe malaria. The WHO classifications, which are most frequently used, have been developed for bedside classification of patients living in high transmission settings, where diagnostics rely mainly on clinical signs and symptoms. Along with this classification the WHO also proposed an epidemiologic and research definition of severe *P. falciparum* considering

both clinical and laboratory findings (table 1). It shall be noted that untreated severe malaria has a very high risk of death. Immediate anti-malarial treatment and intensive care can reduce this mortality rate to <10%[51].

Table 1: *Epidemiological and research definition of severe falciparum malaria* [51]

Impaired consciousness:	A Glasgow Coma Score <11 in adults or a Blantyre coma score <3 in children
Acidosis:	A base deficit of >8 meq/l or, if unavailable, a plasma bicarbonate of <15 mM or venous plasma lactate >5 mM. Severe acidosis manifests clinically as respiratory distress – rapid, deep and labored breathing
Hypoglycemia:	Blood or plasma glucose <2.2 mM (<40mg/dl)
Severe malarial anemia:	A hemoglobin (HGB) concentration <5 g/dl or a hematocrit of <15% in children <12 years of age (<7 g/dl and <20%, respectively, in adults) together with a parasite count >10 000/µl
Renal impairment (acute kidney injury):	Plasma or serum creatinine >265 µl (3mg/dl) or blood urea >20 mM
Jaundice:	Plasma or serum bilirubin >50 µl (3mg/dl) together with a parasite count >100,000/µl
Pulmonary edema:	Radiologically confirmed, or oxygen saturation <92% on room air with a respiratory rate >30/min, often with chest indrawing and crepitations on auscultation
Significant bleeding:	Including recurrent or prolonged bleeding from nose gums or venipuncture sites; hematemesis or melaena
Shock:	Compensated shock is defined as capillary refill ≥3 s or temperature gradient on leg (mid to proximal limb), but no hypotension. Decompensated shock is defined as systolic blood pressure <70 mm Hg in children or <80 mm Hg in adults with evidence of impaired perfusion (cool peripheries or prolonged capillary refill)
Hyperparasitemia	*P. falciparum* parasitemia >10%

Asymptomatic *P. falciparum* infections

Asymptomatic *P. falciparum* infections occurs especially in areas with stable malaria transmission where semi-immune individuals can carry asymptomatic chronic infections with low parasitemia over prolonged periods of time. Even though this infection does not pose a real threat, they constitute an important reservoir of parasites under the radar of the health system. This subsequently enhances the local transmission and poses another obstacle to malaria elimination[55,56].

1.1.7 Laboratory diagnostic

The spectrum of malaria symptoms is wide ranged and has no clinical pathognomonic characteristics. Malaria diagnosis is supported by fever, or history of fever in absence of any other obvious cause. Because of the difficulties to distinguish clinically between malaria and other potential causes of fever, different diagnostic methods can be applied. The gold standard under field conditions is the conventional bright field microscopic examination of a thick blood smear (TBS). There are further microscope based diagnostic tools such as the quantitative buffy coat method and the Partec Rapid Malaria Test. Moreover, different RDTs and a variety of molecular diagnostic methods complement the diagnostic arsenal[57].

Microscopic based diagnosis

Thick blood smear

The gold standard of malaria diagnosis is the microscopic examination of Giemsa-stained TBS. It is cost effective, requires just a small volume of blood, can differentiate malaria species, and quantify parasites with a lower limit of detection of 10-100 parasites/µl (depending on the experience of the laboratory staff). Therefore, it is the most commonly used test in endemic countries. Nevertheless, it relies on the expertise of the reader, the quality of the prepared blood slide and the condition of the microscope[53,57].

Quantitative buffy coat method

Fluorescence microscopes are used to detect acridine orange stained parasites in centrifuged blood. It has a better sensitivity with 5 parasites/µl and requires less trained personal. However, it is unable to differentiate between species and specific equipment is required[57].

Partec Rapid Malaria Test

Prelabelled malaria slides bind intraerythrocytic *Plasmodium* DNA, which can then be visualized under a fluorescence microscope (Partec CyScope®). It is fast, easy to use, requires less experienced personal and due to rechargeable batteries, it is suitable for settings without electric supply. Drawbacks are the specific equipment, difficulties in species differentiation and false positive results[57].

Rapid diagnostic tests

RDTs use immune-chromatographic assays with monoclonal antibodies directed against the target parasite antigen. Results can be obtained within 5- 20 min via a colored test line. Most commonly used RDT only detect *P. falciparum*, but there are already advanced tests available, which distinguish between species. Disadvantages result from the relatively high threshold of 200 parasites/µl, false negative results in presence of high parasitemia (>10%), high costs and variable test result reliability in different environmental conditions. Furthermore, the most commonly used antigen for the RDTs is histidine-rich protein 2, which can be absent in some wildtype parasite strains leading to false-negative results as indicated by recent reports[58]. Therefore, RDTs are limited to situations, when high quality microscopy is unavailable [59].

Polymerase chain reaction

Molecular diagnostic tools such as Polymerase chain reaction (PCR) play an important role in scientific settings to detect resistances, mixed infections, low parasite densities (Up to 0.02 parasites/µl), or asymptomatic carriers. PCR requires highly trained personal, rigorous laboratory sterility, high costs and is relatively time consuming. Therefore, it is not suited for the clinical management of malaria especially in remote areas[53,57].

Non-invasive tests

Further methods are in the developing pipeline, such as biomarkers in human breath, urine malaria tests, or transdermal laser detection of malaria parasites. While these methods are still in their infancy, they yield promising results to serve as future alternative malaria diagnostic tools[57].

1.1.8 Current treatment options

There are a limited number of drugs available to treat or to prevent malaria. They are derived from a restricted set of chemical compounds, which according to WHO classification, can be grouped into 6 categories:

- Quinine and related compounds
- Antifolate combination drugs
- Antibiotics
- Artemisinin compounds
- Miscellaneous compounds (not exhaustive)
- Combination therapy with antimalarials[53]

Uncomplicated malaria

Uncomplicated malaria (excluding infection during first trimester pregnancies) is treated with ACT. In areas of low transmission, an additional single dose of primaquine is recommended[53]. Pregnant women in their first trimester are treated with quinine in combination with clindamycin. Primaquine is one of the few drugs targeting gametocytes and thus reducing transmission. Moreover, it provides radical cure from *P. ovale and P. vivax* by clearing hypnozoites and preventing relapses[60]. However, primaquine may cause fatal hemolysis in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency, which is common in malaria-endemic regions[61].

Severe malaria

For treatment of severe malaria, intravenous or intramuscular artesunate for at least 24 hours is recommended. If parenteral artesunate is not available, artemether is used in preference to quinine. Afterwards, if oral therapy is tolerated, the three days regime is completed with an oral ACT[53].

Chemoprophylaxis

Non-immune travelers visiting malaria endemic areas can be protected by chemoprophylaxis. Especially individuals, who are backpacking or staying overnight in rural areas, are particularly at risk. Atovaquone-proguanil is the most commonly used drug combination for chemoprophylaxis[62].

1.1.9 Current status of anti-malaria drug efficacy

Resistances to anti-malarial drugs have been described for two out of the four species of malaria parasite that naturally infect humans - *P. falciparum* and *P. vivax*[7].

The first safe, effective and affordable anti-malarial drug was chloroquine. It dominated anti-malaria therapy for over two decades. Following a massive monotherapy strategy with chloroquine, development of resistance started to be reported in several areas and spread across Africa in the 1980s leading to a dramatic resurgence of malaria including malaria-related deaths. Today, about 80% of wild isolates are resistant to chloroquine[63]. Although chloroquine is not used in most countries anymore, it remains first line treatment in the Dominican Republic, Guatemala, Haiti, Honduras and Nicaragua[7]. And due to structural modifications, derivates such as amodiaquine and ferroquine[64] are still part of anti-malarial treatment regimens[65].

Today, emerging drug resistances to newer drug classes are threatening their utility, too[10]. Current most effective and rapid acting anti-malarial drugs are artemisinin derivates. To prevent the emergence of resistance by selection of resistant parasite strains, artemisinin is used only in combination with other anti-malaria drug classes, which feature a prolonged half-life. This allows the longer acting partner drug to clear residual parasites, which were not eliminated by artemisinin derivates and therefore limit the selection for resistant parasites strains[10]. Despite these efforts, current treatment options are seriously threatened by emerging of strains with decreased artemisinin sensitivity reported from the Thai-Cambodian border[66,67] and lately also from the Thai-Myanmar border[68,69].

The WHO constantly monitors the efficacy of anti-malarial drug and has established a global database on anti-malarial drug efficacy and resistance containing data on therapeutic efficacy studies for *P. falciparum* and *P. vivax* and, more recently, data from studies of molecular resistance markers[7]. Based on the analysis of available data it has been noted that the efficacy of ACT currently recommended in national malaria treatment policies remains effective with overall efficacy rates of greater than 95%. Nevertheless, it is uncertain if the ACTs will remain effective in the future.

1.2 Vaccine development against *P. falciparum*

The current arsenal of malaria control tools may be sufficient to eliminate the disease in low transmission areas and reduce morbidity and mortality in areas of high transmission, but they are not adequate to eradicate malaria[70]. Therefore, new tools and innovations are needed and a malaria vaccine has tremendous potential for malarial elimination even in high endemic transmission areas[70,71]. Vaccines have proven to be the most cost effective and efficient intervention for public health[72]. They have been utilized in several previous eradication programs such as polio[73], smallpox[74] and measles[75]. Because of the expected potential of a malaria vaccine in the fight against malaria, in 2015 the global health community has called for the development and licensing by 2030 of malaria vaccines with protective efficacy of at least 75%[70].

1.2.1 Classification of malaria vaccine candidates

The first preclinical approaches for a malaria vaccine began in the 1930s[34]. Four decades later Clyde et al. immunized malaria naïve individuals for the first time using irradiated *P. falciparum* sporozoites inoculated via mosquito bite[76]. Given the difficulty to reproduce this method at a large scale and particularly for mass vaccination, it was abandoned. The technological advance observed during the same period allowed the development and a switch to protein based sub-unit malaria vaccine candidates[34]. Despite decades of research, a highly efficient, long lasting anti-malarial vaccine remains elusive. By now, over 5,000 potential *P. falciparum* antigens were isolated and more than 40 malaria vaccine candidates were tested in clinical studies from phase I to III. The current pipeline of clinical development is displayed in figure 4.

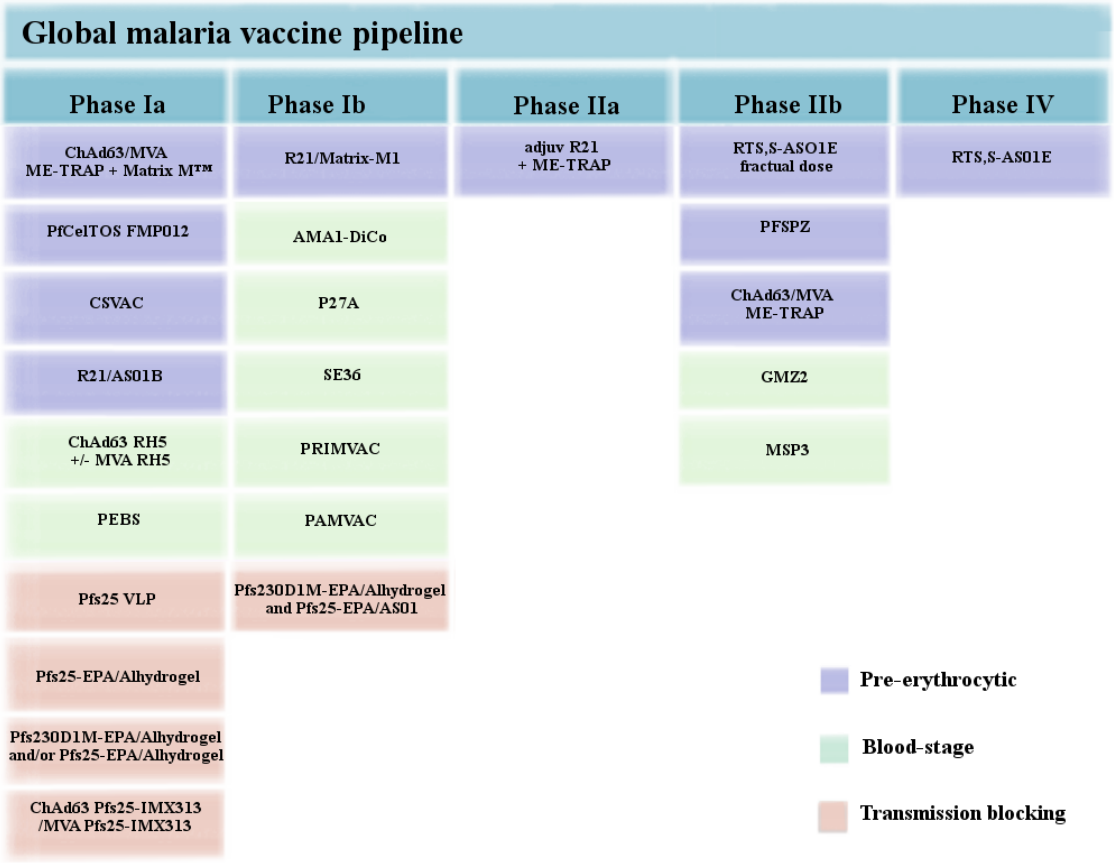
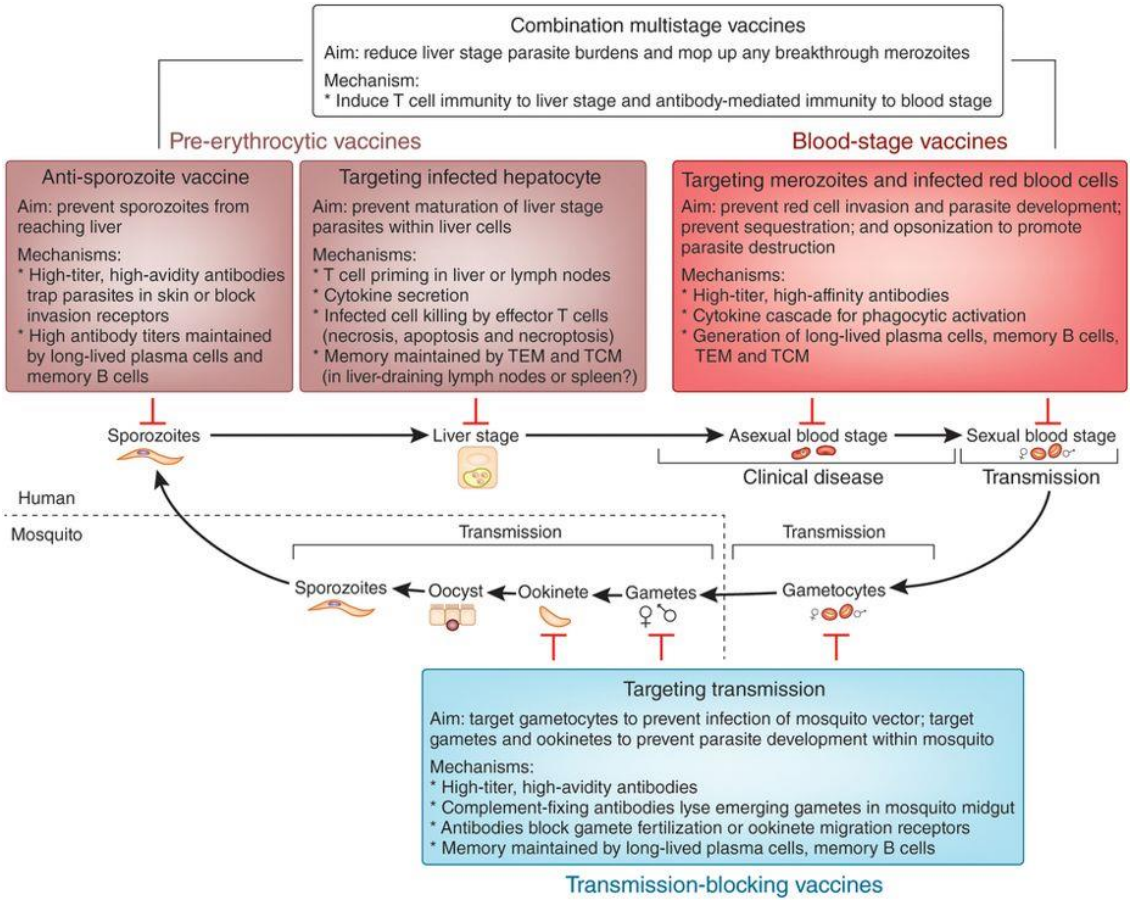


Figure 4: *Global malaria vaccine pipeline created with the data of the WHO malaria vaccine rainbow tables (last updated 17 July 2017)* [77]

Based on the stage of the *Plasmodium spp.* lifecycle they target, malaria vaccine candidates can be divided into four different categories: pre-erythrocytic, blood-stage, transmission blocking vaccines or multistage vaccines (figure 5). Although most of these vaccines are made out of recombinant protein with antigenic properties, also live attenuated whole sporozoites vaccines have witnessed a renewed interest[78].



Riley and Stewart 2013, Figure 3

Figure 5: *Malaria vaccine approaches: Aims and required immune responses. Pre-erythrocytic vaccines intercept parasites before they can reach the blood. They target sporozoites with AB and infected hepatocytes with cytotoxic T cells. This approach aims at sterile protection. In contrast, the blood-stage vaccine acts upon parasites in the asexual and sexual blood stage mainly relying on high titers of AB. While not necessarily offering sterile protection, this approach aims at alleviating clinical symptoms and allowing the development of NAI. Whereas the transmission targeting vaccines prevent parasite development within the mosquito using AB against gametocytes, gametes and ookinetes. This last approach does not protect the individual from clinical malaria, however it prevents transmission and therefore provides protection on a community level. Combination multistage vaccines act on different stages trying to combine the strengths of the singular approaches. TEM, effector memory T cells; TCM, central memory T cells. Reprinted with permission from Nature Publishing Group*[31].

Pre-erythrocytic vaccines

Live attenuated whole sporozoites vaccine

The rationale for this approach is rooted in historical studies conducted in the 1910s showing that immunization of animals with irradiated sporozoites can lead to protective immunity[79]. Translation of these findings were made in human in the 1970s, when human subjects were immunized for the first time by irradiated sporozoites against homologous strains of *P. falciparum.* In this approach the inoculation of parasites was done by the bite of over 1000 infectious mosquitos[76]. Irradiated sporozoites are metabolically active but incapable of completing their life cycle and stay blocked in hepatocytes where they die before reaching the blood. As a consequence, immunized participants do not develop malaria and the immune system is exposed to sporozoites and infected hepatocytes presenting parasite-derived peptides on MHC class I molecules. This induces immune responses to many different epitopes at the same time and subsequently limits the negative effect of antigenic polymorphism and immunological non responsiveness, which is the main obstacles in developing subunit vaccines especially blood-stage vaccines[78]. Despite the promising result obtained during first in human trials, clinical development of live attenuated sporozoites vaccines stopped as it was deemed impractical to use for a malaria control program. Nevertheless, more recently a major breakthrough was made by the production of aseptic, purified and vialed *P. falciparum* sporozoites by SANARIA which can be inoculated intravenous to potential recipients[80].

Several clinical trials have been or are currently conducted to assess the tolerability profile and the efficacy of live attenuated whole sporozoites vaccines in Europe and Africa. Overall, they show that life attenuated sporozoites vaccines are generally safe and can reach 80-100% efficacy in homologous vaccine and challenge trials[80].

Of note, vaccine efficacy was much lower (30-50%) in semi-immune study subjects under natural exposure[81]. Reasons for the lower efficacy are currently under investigation. Moreover, large-scale production according to good manufacturing practices, transportation and storage in liquid oxygen are still posing obstacles. Further research has to conclude, whether these obstacles can be overcome.

Besides the use of irradiation, *Plasmodium spp*. sporozoites can also be attenuated through genetic modification or by chemoprophylaxis in a course of *Plasmodium spp*. sporozoites inoculation.

Recombinant pre-erythrocyte malaria vaccine

Among the recombinant pre-erythrocyte malaria vaccines RTS,S has been the most promising. It is the only malaria vaccine candidate reaching a phase III efficacy trial. Result of this phase III trial indicated a good tolerability profile and an efficacy ranging from 20 to 40%[82]. The vaccine received a positive scientific opinion by the European Medicines Agency in 2015[83] and was recommended by the WHO for a pilot implementation program in Africa, which is currently ongoing in Ghana, Kenya, and Malawi[84]. RTS,S was engineered using genes from the pre-erythrocytic circumsporozoite protein (CSP) of the *P. falciparum* malaria parasite and a viral envelope protein of the hepatitis B virus (HBsAg) adjuvanted with AS01. Infection is prevented by inducing humoral and cellular immunity with high antibody titers blocking the parasite from infecting the hepatocytes. Other pre-erythrocytic recombinant malaria vaccines have been or are currently under clinical development but none has provided considerably better results.

Present pre-erythrocytic approaches may diminish, but not eliminate, the risk of infections with *P. falciparum*. Reaching an efficacy of up to full protection in a malaria endemic setting remains elusive. That leaves the fraction of immunized population at risk, where, despite immunizations, *P. falciparum* slips through pre-erythrocytic protection, and reaches blood-stage. Without a blood-stage immunity, a parasite reaching blood-stage is likely to induce clinical malaria infection. Moreover, any intervention with partial efficacy bears the risk of a rebound effect as it slows down the development of NAI as demonstrated with RTS,S[85]. This indicates that any malaria vaccination approach aiming at minimizing long-term morbidity and mortality should ideally implements a blood-stage component to control the erythrocytic schizogony.

Asexual blood-stage vaccines

Asexual blood-stage vaccines (BSV) aim to directly reduce malaria morbidity and mortality since the clinical features of the disease are caused by the asexual erythrocytic schizogony. The feasibility of immunization with *Plasmodium spp*. blood-stage antigens is rooted in the observation, that the NAI to the parasite is partly mediated by immunoglobulin against blood-stage *Plasmodium spp.* antigens. It was demonstrated that the transfer of serum of lifelong malaria-exposed immune adults could be used therapeutically to ameliorate disease symptoms and control parasite density of malaria patients[40,41]. However, the identity or pattern of the protective immunoglobulin is still to be determined, since during the blood-stage the immune response to the parasite appears to be complex and a vast variety of epitopes are expressed[35]. In general, a suitable antigen to be incorporated in a BSV candidate should elicit a sufficient immune response, while being conservative enough to cover an adequate number of genetically distinct parasite strains in order to be efficacious and minimize the risk of inducing vaccine escape mutants. It is highly likely that the vaccine-induced immunity could be boosted by natural infection, and therefore maintained naturally over a long period of time. While not inducing sterile immunity, it subsequently allows the development of NAI. Therefore, a BSV may not only be used as stand-alone product, but also in combination with a pre-erythrocytic vaccine in a sense that any parasite, which was not neutralized through the pre-erythrocytic immunity would be targeted by the BSV.

The current repertoire of BSV is made of recombinants of *Plasmodium spp*. proteins. A leading candidate of the group of asexual blood-stage, GMZ2, has shown to be most promising and has reached a phase IIb multi-center trial.

Malaria transmission-blocking vaccine

Malaria transmission-blocking vaccines (TBV) primarily aim to reduce malaria transmission by interrupting the sexual, and sporogonic stage of the parasitic life cycle within the mosquito. Unlike pre-erythrocyte and BSV, TBV do not prevent infection, reduce parasite load or prevent the disease in its recipient[86]. However, by stopping *Plasmodium spp*. transmission, TBV have the potential to reduce malaria morbidity, decrease the size of the parasite human reservoir and may even lower the basic reproductive number below one, leading to local malaria elimination[87]. The use of TBV

in combination with an anti-malarial drug (i.e. in mass drug administration programs), or TBV antigens as a component in a multivalent vaccine could also help to contain the occurrence of drug and potential vaccine resistances[88]. From an evolutionary perspective, the selection pressure exerted on the parasite during its mosquito stage is thought to be lower than during the human development life cycle. As a consequence, it is assumed that *Plasmodium spp.* sexual stage antigens are less polymorphic making it easier to develop vaccines which exert cross-species immunity [34,89]. There are currently few antigens with the potential to be used or being used for the development of TBV. So far, only a limited number of TBV candidates are currently under clinical development (AnAPN1, Pfs230, Pfs48/45, Pfs25)[86]. A clinical phase I study regarding the most advanced TBV candidate Pfs25 shows promising results, while also raising safety issues[89].

Multistage malaria vaccines

Combining pre-erythrocytic, erythrocytic and even TBS give the advantage of targeting multiple stages of the parasite life cycle. In this approach blood-stage antigens are to induce an immune response targeting parasites, which could not be neutralized by the protection induced by pre-erythrocytic immunization[90]. This can give the advantage of limiting vaccine resistance. Downsides of multistage malaria vaccine exist and are mainly associated with an increase in manufacturing costs[91] and possible antigen interference leading to diminished immune responses against individual antigens[92].

Vaccines against pregnancy-associated malaria (PAM)

Especially during primigravidae women are prone to malaria, as already described in section 1.1.1 (Epidemiology). The rapid reestablishing of NAI during subsequent pregnancies indicates a specific immune mechanism for PAM[93]. The sequestration of the erythrocytes to maternal side of the syncytiotrophoblast is mediated by VAR2CSA, a conserved variant of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). The titer of anti-VAR2CSA antibodies correlates with reduced risk of delivering low-birthweight babies[94]. This subsequently has led to PfEMP1 as the first vaccine candidate for PAM in preclinical development[34,95].

1.2.3 GMZ2: A blood-stage malaria vaccine

GMZ2 targets the asexual blood-stage and is a recombinant fusion protein containing fractions of *P. falciparum* antigens. The N-terminal non-repeat region of the glutamate-rich protein (GLURP-R0, amino acids 27-500) is fused to the C-terminal region of merozoite surface protein 3 (MSP3, amino acids 212-380) and expressed in *Lactococcus lactis*[96]. The production system with *Lactococcus lactis* was chosen, because the gram positive bacterium is well described, has been consumed by humans for centuries, has low immune stimulation potential, does not produce endotoxins, and can efficiently secrete GMZ2 into the culture supernatant[96]. Endogenous $GLURP_{27\text{-}500}$ is a conservative[97] major B cell epitope expressed in the pre-erythrocytic and erythrocytic stage[98]. $MSP3_{212\text{-}380}$ is a conservative part of the otherwise highly polymorphic MSP3[99,100]. It is one of the first vaccine candidates identified by analyzing therapeutic immunoglobulin preparations[41,101] and immune-epidemiological correlations studies[102]. GMZ2 itself is a sufficient presentation of epitopes of GLURP and MSP3 and elicits higher immunogenicity compared to the administration of the single protein MSP3 or GLURP[96].

Immuno-epidemiological studies

There are several BSV candidates in clinical development. However, the majority of studies conducted so far have shown disappointing results. Either the protection induced by the vaccine candidate is too low (falciparum malaria protein 1, MSP1[103], Apical membrane antigen 1 (AMA-1)[104]), or it is limited to the vaccine strain (MSP1 and MSP2, ring-infected erythrocyte surface antigen (RESA)[105], AMA-1[106]). Among the known repertoire of antigens expressed by the parasite during the blood-stage, MSP3 and GLURP are deemed as promising vaccine candidates. This is mainly based on epidemiological and laboratory data indicating an association between MSP3 and GLURP antibody levels and clinical protection against malaria[101,107–110]. Indeed, individuals in malaria endemic countries develop antibodies against MSP3 as well as GLURP[110] under natural infection. These antibodies seem to play an important role in NAI and are associated with protection[109,111,112]. This later observation is of particular interest, as it suggests that vaccination with GMZ2 cannot only induce protection but may also be boosted by natural infection.

Although the mechanism of protection to *P. falciparum* elicited by the vaccine is not yet fully understood, it has been shown that the vaccine induce specific cytophilic IgG_1 and IgG_3 are associated with protection against malaria[109,111]. Evidence indicates that vaccine specific antibodies can also mediate opsonic phagocytosis of merozoites by binding Fcγ-receptors of neutrophils. If FcγRIIA and FcγRIIIA receptors on monocytes are activated by cross-linked IgG bound to parasite antigens, soluble factors are released and ADCI is initiated. This appears to be the predominant effector mechanisms rather than direct growths inhibition[42,113–116]. Among all proposed surrogate markers considered, ADCI is the method showing the closest association with clinical protection[117] and is an accepted method to validate vaccine candidates[118]. GLURP[119], MSP3[101], and GMZ2[118] have shown to induce good ADCI reactions. Especially cytophilic MSP3 antibodies have a strong capacity to induce ADCI[120].

Pre-clinical assessment

GMZ2 has been evaluated in several pre-clinical studies. GMZ2 immunized mice showed higher levels of anti-GLURP and MSP3 compared to mice injected with single GLURP, MSP3 or both antigens[96]. Moreover, an in vitro parasite-growths inhibition was demonstrated using mouse anti-GLURP-MSP3 IgG in cooperation with human monocytes[96]. The tolerability, immunogenicity and efficacy were further evaluated during a study in *Saimiri sciureus* monkeys. The study achieved partial protection with GMZ2 upon challenge with *P. falciparum* and demonstrated that anti-GMZ2 IgG can be boosted by challenge [108]. This indicates that the vaccine protection might be boosted by natural infection.

Clinical development

The first-in-man phase I trial clinical study in healthy malaria naive adults took place in Tübingen in 2006 to access safety, tolerability, and immunogenicity in humans. Since the study was successful[121], the next trial moved from malaria naïve participants to semi-immune adults. This was done to access whether the results can be reproduced in a malaria endemic surrounding. The phase I trial in Gabon in 2007 confirmed the good tolerability profile and proved that the pre-existing immune response can be boosted[122]. Consequently, the development proceeded to the target population of healthy African children (2008). While achieving promising results, the study had not the power to access

vaccine efficacy[123]. Thus, a large phase IIb multicenter study in Ghana, Burkina Faso, Gabon and Uganda was conducted in 2010. Although the study showed a good tolerability profile and robust immunogenicity, the vaccine efficacy with 13.6% (95% CI: 3.6%, 23%) was to low compared to what is required for a substantial impact[124]. However, at all study sites vaccine efficacy was similar, suggests a pan-reactive vaccine, which is not limited by strain specific immunogenicity. Moreover, a significant relationship between the immunogenicity and vaccine efficacy could be established. This indicates, that another GMZ2 formulation, which elicits higher antibody titers could significantly increase vaccine efficacy.

During the pre-clinical studies GMZ2 was adjuvanted with different types of adjuvants, which had a high impact on the level of antibodies elicited[108]. This led to the assumption that GMZ2 given with a different adjuvant could still meet the requirement of the preferred product profile of the WHO for malaria vaccine[125].

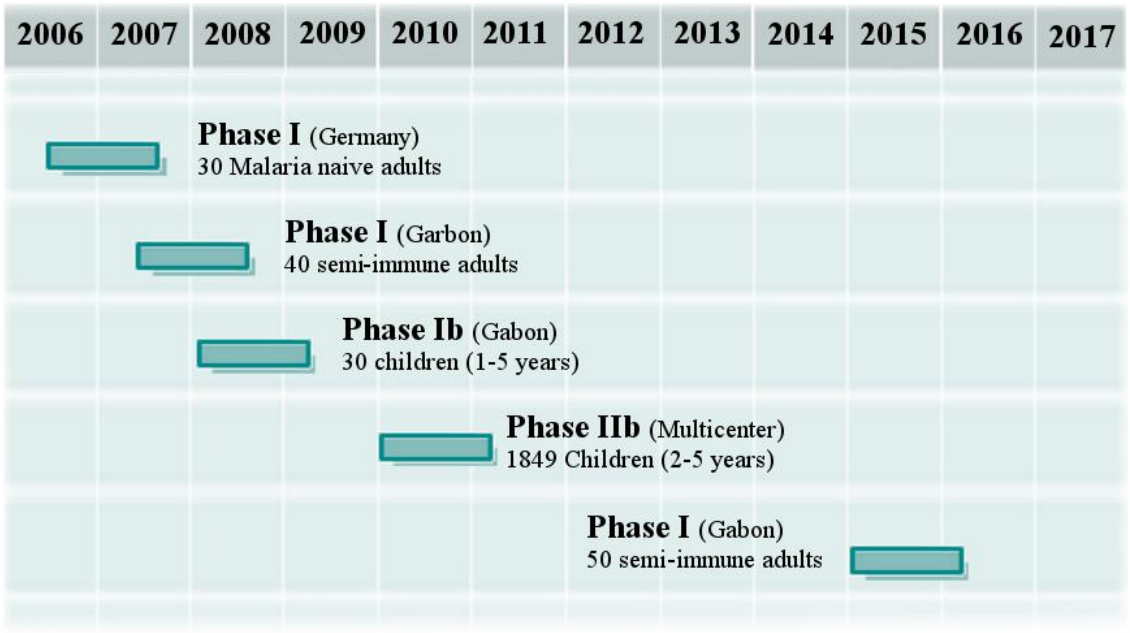


Figure 6: *Clinical trials of GMZ2*

Adjuvant selection

Many subunit vaccines require an adjuvant to be immunogenic. While most malaria vaccine trials used to start with aluminum salts as the adjuvant, evidence rises that especially subunit vaccines need a more potent delivery system to be successful. A good adjuvant preserves the conformational integrity of the vaccine antigen and delivers it to immune effector cells. There, it presents the vaccine antigen at a bigger size or other mechanisms that induce a response that facilitates the recognition and uptake through antigen presenting cells (APC). Simultaneous activation of distinct PRR of the same APC induces a higher proinflammatory response and the upregulation of necessary cytokines[126]. This subsequently leads to a stronger initiation of innate and often to a better adaptive immunity. Additionally, an adjuvant generates a depot of antigen and can mediate prolonged and controlled release of antigens supporting long-lasting immune responses[47]. Further, it influences not only the magnitude of elicited immunogenicity but also the avidity of antibodies and can broaden the vaccine response against heterologous pathogen strains[127]. Different adjuvants elicit distinct immune responses depending on the evoked cytokines and on promoted T-helper (T_H) cell subsets.

Alhydrogel, an aluminum hydroxide suspension, was chosen in previous GMZ2 trials because of its good tolerability profile, since the final target population of a blood-stage malaria vaccine are children in Sub-Saharan Africa. It is one of the most common, cost-effective adjuvants, already used for over 80 years and has an extensive track record of safety and tolerability[128]. The downside is the inability to induce a T_H1 immune profile promoting INF-γ, and IL-12, which appears to be crucial for protection against malaria[129,130]. Moreover, in a pre-clinical study was shown, that GMZ2 combined with Alhydrogel elicited lower antibody response than combined with different adjuvants[108,131]. Furthermore, the leading malaria vaccine candidate RTS,S showed little efficacy when adjuvanted with Alhydrogel[132].

These observations motivated to change the type and nature of adjuvant used for the GMZ2 vaccine and to investigate a formulation with the Cationic adjuvant formulation 01 (CAF01). CAF01 is developed by Statens Serum Institut and contains two components: dimethyldioctadecylammonium (DDA) cationic liposomes and α,α'-trehalose 6,6'- dibehenate (TDB). The DDA is a cationic quaternary ammonium salt supporting the uptake and presentation of the vaccine antigens in the appropriate subset

of APC. The TDB is a glycolipid, further described as synthetic analog of trehalose 6,6'-dimycolate (TDM or cord-factor)[133]. It activates the APC through syk-Card9 signaling pathway[134]. Being first developed within a program to improve vaccines against tuberculosis (TBC)[135], it was also tested for HIV[136,137] and malaria[131]. There, it showed not only potent immune-enhancing properties on humoral and cellular immune responses, but also a good safety and tolerability profile. A recent study including a vaccine candidate against TBC confirmed long lasting immunity[135]. Especially the elicited T_H1 mediated immune response is promising for a BSV adjuvant. In direct comparison against Alhydrogel in a pre-clinical trial it showed superior immunogenicity and protection[131].

1.3 Helminth infection in humans and their impact on malaria vaccines

1.3.1 Potential factors capable of impairing vaccine efficacy and immunogenicity in malaria endemic areas

Among the various challenges in vaccine development, one has to account for the special situation in endemic countries. Frequently, vaccine candidates are first tested for tolerability and immunogenicity in populations of high income and non-endemic countries, which can have a fundamentally different immune profile compared to the population in endemic countries[138]. Therefore, it is sometimes difficult to reproduce a result of efficacy trials conducted in non-endemic countries when they are conducted in endemic countries[139–141]. This particularly applies to vaccine candidates for parasitic diseases and has been shown in various studies; most recently, by using controlled human malaria infection (CHMI) to assess vaccine efficacy in both European and African study subjects receiving the *P. falciparum* sporozoite Sanaria vaccine (PfSPZ Vaccine)[81].

There are several factors that may impair the efficacy of a vaccine in endemic areas such as: maternal antibodies[142,143], malnutrition and inadequate micronutrient intake[144,145], immune tolerance and co-infections[146–156]. The impact of co-infection with helminths on vaccine immunogenicity will be detailed further in the following section.

1.3.2 Epidemiology of helminths

Helminth infections occur, *inter alia*, in stable endemic malaria transmission areas. The most ubiquitous species, which share the same spatial distribution as *P. falciparum* in sub-Saharan Africa are the soil-transmitted helminths with 1.5 billion people infected[157]. Hookworm infections occur throughout almost the whole continent, whereas *Ascaris lumbricoides* (*A. lumbricoides)* and *Trichuris trichiura (T. trichiura)* are more frequent in equatorial regions[158,159]. Other helminths such as *Schistosoma* are often focal distributed, owing to spatial heterogeneities in human behavior and the presence of water-bodies[159,160]. Around 102.3 million people were treated in 2017[161]. With an even more restricted incidence *Loa loa* (*L. loa)* infects around 10 million people in central and western Africa. It is mainly endemic in ten tropical countries. Gabon and Equatorial Guinea are high risk areas[162].

Because of the geographical overlap between helminths and malaria, humans living in malaria endemic countries are usually co-infected with or exposed to helminths. This can have an impact on the immunogenicity and efficacy of malaria vaccine candidates.

1.3.3 Helminths induced immune regulation and its impact on vaccine elicited immune response

By comparison to other pathogens, helminths are large macro-parasites. Because of their long lifespan (ranging from 1 to 8 years depending on the species), helminth infection of the human host usually results in a chronic infection that can last years or decades if not treated. Parasitic helminths have developed a set of mechanisms to escape the host immune system. Chronic infections with helminth have been shown to induce a skewed T_H2 immune response, which is marked by the involvement of CD4+ T_H2 cells subset, the release of T_H2-type cytokine such as IL4, IL5, IL8, IL10, and IL13, the secretion of IgE, and a subsequent expansion of eosinophil effector cells. Concomitant to the T_H2 type response, chronic helminth infection also lead to a dampening of the immune system through the involvement of T_{reg} and the subsequent secretion of immune-suppressive cytokines such as Il-10 and TGF-ß[163–165]. Moreover, T cells may become exhausted and undergo apoptosis[166]. This induces a state of immune hypo-responsiveness that benefits the parasite[159,167] but may also be of advantage for the human host as it prevents an overwhelming pro-inflammatory T helper 1 (T_H1) response and subsequent organ damage[167].

As a disadvantage, the induced hypo-responsiveness can also affect reaction to other (bystander) antigens with the potential to alter vaccine induced immunogenicity[167,168]. Indeed, an effective vaccination against most bacterial and viral pathogens often requires a strong T_H1 immune response. Impairment of vaccine-induced immunity was first discovered for oral vaccines such as polio[146], rotavirus[149,169], or cholera[147,148], and subsequently also for vaccines administered through parenteral routes such as TBC[151,153], tetanus[155] and malaria vaccine candidates[138,168,170,171]. These findings suggest that an effective vaccine for humans living in helminth endemic areas might need a modified adjuvant eliciting a more T_H1 polarized immune response, or the patient should be treated for parasitic infections before vaccination. However, more data on the impact of helminths infections on malaria vaccines is needed.

1.4 Aim of this thesis

The aim of this thesis is to assess the safety, tolerability and immunogenicity of GMZ2 adjuvanted with either aluminum hydroxide or CAF01 in lifelong malaria exposed Gabonese subjects. It reports a clinical phase I study that included CHMI to assess efficacy. Results of the CHMI are not reported. Moreover, as the study area is also endemic for helminth infection, the impact of helminth infection on GMZ2-induced immunogenicity is investigated.

The main objective is to assess the tolerability profile of GMZ2-CAF01 compared to GMZ2-Alhydrogel and a control vaccine (rabies vaccine) by assessing the number and severity of local and systemic adverse events (AE), the number and severity of serious AEs, and the causal relationship between AE occurrence and vaccination.

The second objective is to compare the immunogenicity of GMZ2-CAF01 against GMZ2-Alhydrogel by measuring the humoral response, and to assess the impact of helminth infection on the elicited immunogenicity.

The third objective is an explorative analysis of the relationship between AEs and the magnitude of immune response.

2. Methods

2.1 Study design

The study was designed as a phase I vaccine trial entitled “A randomized, controlled, double-blind, single-center phase I clinical trial to evaluate the safety, tolerability, immunogenicity and efficacy of CAF01 and aluminum hydroxide as adjuvants for the malaria vaccine candidate GMZ2 in healthy adult African volunteers”. The trial protocol can be accessed as part of the puplication[172].

2.1.1 Study period

The trial started with the first vaccinations on the 20th of April 2015 and ended with the last follow up visit on the 22th November 2015. The study period covered the end of the rainy season in spring, the dry season in summer and the start of the second rainy season in autumn.

2.1.2 Study site

The trial was conducted at the Centre de Recherches Médicales de Lambaréné (CERMEL), Gabon (figure 7). CERMEL originated out of the Medical Research Unit of the Albert Schweitzer Hospital, which was founded in 1981 as an integral part of the hospital. In 2011 the research unit was transformed into an independent non-profit organization and renamed into CERMEL. It has a robust track record on clinical trials and decades of experiences with conducting studies according to the “International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice (GCP)” guidelines in the area of Lambaréné. The research activities of CERMEL include various epidemiological and interventional trials on tropical diseases. Amongst them studies on anti-malarial drugs (phase I-III), CHMI trials, studies of several malaria vaccine candidates (phase II and III) and, precursor GMZ2 trials (phase I and II) have been conducted.

Figure 7: *Medical research unit (CERMEL) (left), Lambaréné[173] (right)*

Lambaréné itself is a semi urban town located just below the equator in the central African rainforest (figure 8). It is crossed by the river Ogooué, one of the grand rivers of Central Africa. With a population of around 25,000 Inhabitants, it is the capital of Moyen-Ogooué, which is one of the nine provinces of Gabon. Gabon has a population of approximately 1.5 million inhabitants with 80% concentrated in the urban areas. There are around forty different ethnic groups. Bantu Tribes are the most common ones including Fang, which represents about 30% of the whole population[174].

Figure 8: *Map of Gabon from the Blue Marble collection of NASA adapted for this thesis*[175]

Climate

The average annual temperature in Lambaréné is 26.6°C, the humidity ranges between 80 to 84% and the precipitation is of 257mm divided on two rain seasons February to May and October to November[176,177] as shown in figure 9 and 10.

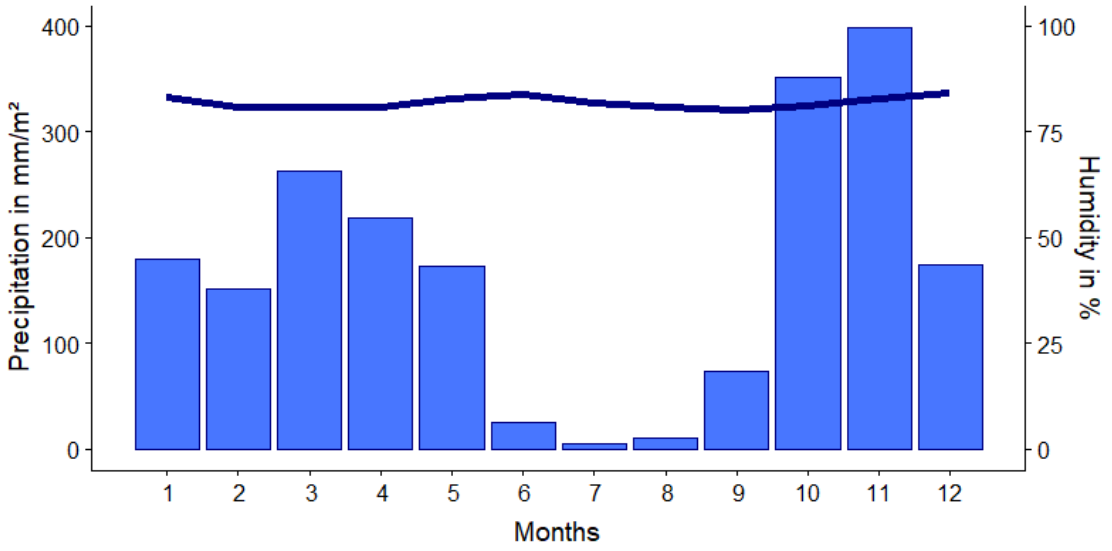


Figure 9: *Precipitation and humidity in Lambaréné. The light blue bars represent the precipitation in each month, whereas the humidity is displayed by a dark blue line*[176,177].

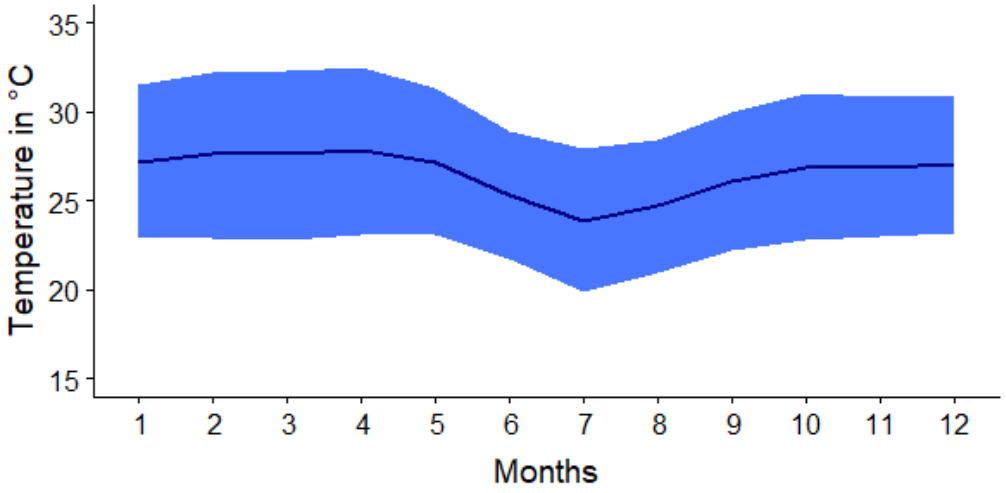


Figure 10: *Temperature in Lambaréné. The dark blue line represents the average temperature during the year. The light blue area displays the temperature range*[176,177].

Endemic diseases

The area is endemic for malaria and various helminths infections. With an entomological inoculation rate of about 50 infective bites per person per year and little seasonal changes malaria is hyper endemic in Lambaréné and surroundings[178,179], although incidence decreased over the last decades. The disease represents one of the major health problems of Gabon. Most infections are caused by *P. falciparum* (95%), whereas *P. malariae* and *P. ovale* play a less important role. *P. vivax* does not occur. The prevalence for parasitemia in afebrile adult males in the area of Lambaréné is 52%, 40% being submicroscopic malaria infections[180]. Severe malaria occurs nearly exclusively in children. At the age of 2-12 years, children experience ~ 1.5 malaria attacks per year in average, with a large individual variability[181]. Concerning anti-malarial drug resistance, the prevalence of chloroquine-resistant genotypes is continuously high (89%)[182]. Moreover, prevalence of the wild-type allele N86Y of the *P. falciparum* multidrug resistance 1, which is associated with decreased lumefantrine sensitivity, is increasing[183].The predominant vectors for malaria transmissions in Lambaréné are *Anopheles gambiae* and *Anopheles moucheti.*

Helminths are also highly endemic in Lambaréné and the main species are *Schistosoma haematobium* (*S. haematobium), L. loa* and *Mansonella perstans* as well as intestinal helminths species such as *A. lumbricoides*. A survey of the minister of health which included 418 subjects living in different villages in the in Moyen-Ogooué province showed a general prevalence of 10.1%, for urinary schistosomiasis, 2.4% for intestinal schistosomiasis and 38.5% for soil-transmitted helminths[4]. Furthermore the prevalence of filaria infections was found to be 26.4% for *L. loa* and 14.6% for *Mansonella perstans* [184]. Co-infection with both helminth and/or malaria usually start at young age with a prevalence of 6% in 1-5 years old children and reaches the peak of infection up to 55% in school-age (own unpublished data).

2.1.3 Study participants

Study participants were healthy, male Gabonese adults living in Lambaréné with a life-long exposure to malaria. The inclusion and exclusion criteria were chosen in order to minimize potential risks for the participants. The criteria ensure the absence of diseases such as blood disorders, chronic illnesses such as Hepatitis C, Hepatitis B or HIV, immune suppression, cardiovascular diseases or inflammation. Moreover, these evaluated neurological and psychiatric risk factors as well as drug abuse. Additionally, these criteria aimed to assess whether the participant received any other vaccine or investigational product in the recent past.

Inclusion criteria

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

Exclusion criteria

- Receipt of an investigational product in the 30 days preceding enrollment, or planned receipt during the study period.
- Prior receipt of an investigational malaria vaccine.
- Immunization with more than 3 other vaccines within the past month.

- Any confirmed or suspected immunosuppressive or immunodeficient state, asplenia, recurrent, severe and chronic (more than 14 days) infections, immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed).
- Use of immunoglobulins or blood products within 3 months prior to enrolment.
- Sickle cell disease or any clinically relevant blood disorder.
- Any clinically significant abnormal finding on biochemistry or hematology blood tests, urine analysis or clinical examination.
- Abnormal electrocardiogram on screening: pathologic Q wave and significant ST-T wave changes, left ventricular hypertrophy, non-sinus rhythm except isolated premature atrial contractions, right of left bundle branch block, advanced A-V heart block (secondary or tertiary).
- A QT/QTc interval > 450 ms.
- History of seizure.
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
- History of serious psychiatric condition that may affect participation in the study.
- Any other serious chronic illness requiring hospital specialist supervision.
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 60 g per day.
- Suspected or known injecting drug abuse in the 5 years preceding enrollment.
- Subjects unable to be closely followed for social, geographic or psychological reasons.
- A history of allergic disease or reactions likely to be exacerbated by vaccine administration.
- Contraindications to the use of the first-line anti-malarial medications: artemether/lumefantrine or atovaquone/proguanil.
- Positive for hepatitis B surface antigen (HBs-antigen).
- Seropositive for hepatitis C virus (antibodies to hepatitis C virus).
- Positive HIV test.

- Any other significant disease, disorder or finding which, in the opinion of the Investigator, may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.

2.2 Clinical procedures

2.2.1 Informed consent procedure and Screening

Screening of potential study participants was conducted in two steps. As a first step, local authorities were informed about the clinical trial and approval was obtained. Then, screening process was started by providing information about the study to the communities. This was done by experienced field workers under supervision of physicians.

Interested potential participants were invited to the research facilities, where they had a meeting with a study physician. During this meeting the study physician explained the study objective and procedures in a language that was adequate for potential study participants. Additional questions were answered *ad libidum*. Participants were given a synopsis of the study to read at home and were re-invited the next day.

Participants, who expressed their willingness to participate in the study, signed an informed consent and were screened for trial eligibility by study physicians. During the screening visit, a thorough clinical examination was performed along with laboratory analysis. The clinical examination consisted of physical examination, electrocardiography, measurement of blood pressure, heart frequency, temperature, and body weight as well as body height. For laboratory assessments blood and urine samples were collected to determine the liver (ALAT, ASAT, LDH) and renal function (urea, creatinine, urine dip stick). A blood count was also realized to determine the level of erythrocytes, HGB concentration, hematocrit as well as the platelet count. A total and differential leukocyte count was performed in search of infection markers and blood disorders. Suitable participants were enrolled, obtained a study identification number, and were listed on the screening log.

2.2.2 Randomization

A computer-generated randomization list was used to allocate participants to the intervention groups and distributed in sealed envelopes. One of the lists was given to the local safety monitor to be kept in case that an emergency requires unblinding and the other to the vaccination formulation team. In case of a drop out of participants before the first vaccination, they were replaced by the next eligible participants on the screening log.

2.2.3 Study vaccine

CAF01 and aluminum hydroxide adjuvant were manufactured by Statens Serum Institut (SSI, Copenhagen, Denmark), whereas the lyophilized antigen (GMZ2) was provided by Novasep (Lyon, France). Investigational products were made according to good manufacturing practices. Locally purchased Verorab Rabies vaccine (Sanofi Pasteur, Lyon, France) served as the comparator vaccine. GMZ2 was shipped on dry ice from Novasep (Gosselies, Belgium) to CERMEL by World Courier Belgium n.v./s.a. (Zaventem, Belgium). The temperature was kept between - 20°C to -90°C as monitored with TempTale 4 USB (Sensitech cold chain visibility, Beverly, USA). CAF01 and aluminum hydroxide were shipped by Statens Serum Institut from Copenhagen to Libreville, where the investigational products were received by CERMEL staff and transferred to Lambaréné. The temperature was monitored with Libero CB loggers (Sensitech cold chain visibility, Buchs, Switzerland). At CERMEL the adjuvants were stored at 2 to 8°C until further use. At the study site, vaccine preparation was under the responsibilities of the vaccine manager. Vaccine preparation was done according to a standard operation procedure following good manufacturing practice guidelines. Preparation was done in a sterile flow hood and consisted in reconstitution of the lyophilized GMZ2 antigen with the corresponding adjuvants. The following adjuvant concentrations were used: 625µg DDA and 125µg TDB for each CAF01 based formulation and 0.85mg $Al(OH)_3$ for each dose of aluminum-based vaccine formulations. GMZ2 was used at the concentration of 30µg and 100µg. After reconstitution three different vaccine formulation were obtained; 100µg GMZ2 + 0.85mg $Al(OH)_3$, 30µg GMZ2 + 625/125µg CAF01 and 100µg GMZ2 + 625/125µg CAF01. The comparator vaccine was reconstituted along the instructions of the supplier. The vaccine

powder was reconstituted with adjuvant to obtain the respective dose in 0.5 ml. The vaccines were prepared on each vaccination day (0, 28, and 56), stored at 2 – 8°C in a fridge and used within 8 hours by intramuscular injecting in the deltoid muscle. Before each vaccination a symptom-directed physical examination with inspection of the vaccination site was performed. An advanced-life-support trained physician was present during the vaccination process.

Allocation concealment was maintained by different measures. The vaccines were reconstituted by a pharmacist, who obtained the randomization list in a sealed envelope directly from the study coordinator. Administration of the vaccines was performed by special trained nurses in a separated vaccination room. None of these persons had any other responsibilities within the trial. They had no further contact to the participants, laboratory team or sample management. Access to the vaccination room and vaccination procedure was prohibited for any other member of the study team.

2.2.4 Vaccination schedule

Each participant received three doses of either the investigational vaccine or the comparator vaccine 28 days apart. Vaccine administration was done in alternating deltoid muscles. Depending on the vaccine formulation the study participants were split into four groups:

- Group A: Comparator vaccine (Rabies) n = 8
- Group B: 100µg GMZ2 +0.85mg Al(OH)3 n = 12
- Group C: 30µg GMZ2 + 625/125µg CAF01 n = 8
- Group D: 100µg GMZ2 + 625/125µg CAF01 n = 22

All study participants were vaccinated within 4 days. For safety concern the number of vaccinated subjects steadily increased from the first day (6 participants) to the fourth day (17 participants) as captured below.

- Study day 0: A (n = 2), B (n = 3), C (n = 1)
- Study day 1: A (n = 2), B (n = 3), C (n = 5), D (n = 1)
- Study day 2: A (n = 2), B (n = 3), C (n = 1), D (n = 10)
- Study day 3: A (n = 2), B (n = 3), C (n = 1), D (n = 11)

2.2.5 Follow up

After each vaccination the participant was kept under direct surveillance for 30 minutes at the research facilities under close monitoring by a study physician. Participants were re-invited to the research facilities on day 1, 7 and 14 following each vaccination as well as on day 28 following the third vaccine administration. During these follow ups, they underwent a basic clinical examination (vital signs and tympanic temperature) and were interviewed to solicit AEs. On day 2, 4 and 6 post vaccination study participants were actively followed up at home by field workers and physicians. This aimed to ensure safety of the participants and collect data on solicited local and systemic AE. Moreover, participants were encouraged to visit the research facility and report occurrence of any health issue. Additionally, a 24-hour operated telephone line was available to contact the study team at any time. At the CERMEL a study nurse and a study physician were available all times.

Blood was collected at different time points for routine analysis (day 0, 7, 14, 28, 35, 42, 56, 63, 70, and 84) and in order to assess the humoral and cellular mediated immune response to the vaccine candidate (day 0 and 84). Stool samples were collected for the detection of helminths infections at the screening visit and at day 84. A summary of study procedures is given in table 2.

Table 2: *Study procedures (* Screening visit, additional actions performed: Informed consent, full medical history, review of vaccinations, assessment of in/exclusion criteria, virology (HIV, Hepatitis B, Hepatitis C) **First vaccination, additional actions performed: Randomization, Assignment of identifier and Supply of identifier card, ***Laboratory analysis includes complete blood count, AST (Aspartate transaminase), ALT (Alanine transaminase), and creatinine)*

Day	**SCR***	**0**	**1**	**2**	**4**	**6**	**7**	**14**	**28**	**29**	**30**	**32**	**34**	**35**	**42**	**56**	**57**	**58**	**60**	**62**	**63**	**70**	**84**
Vaccinations		I**							II							III							
Visits at CERMEL	x	x	x				x	x	x	x				x	x	x	x				x	x	x
Home visits				x	x	x					x	x	x					x	x	x			
Adverse event review		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical examination	x	x							x							x							x
Blood for basic laboratory analysis***	x	x					x	x	x					x	x	x					x	x	x
Serum for Immunology		x																					x
Stool and Urine for helminths infection	x																						x

Adverse Events

An AE is defined by the WHO as follows: "Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product"[185]. For marketed medicinal products the definition is extended to failure of produce expected benefits, abuse or misuse.

The severity of AE was assessed with the grades depicted in table 3. For the solicited AEs gradings were further defined as described in table 5 and 6.

Table 3: *Grading of severity of AE*

Severity grading		Explanation
1	**Mild**	No effect on activities of daily living
2	**Moderate**	Some interference with activity not requiring medical intervention
3	**Severe**	Prevents daily activity and requires medical intervention
4	**Life-threatening**	Hospitalization; immediate medical intervention or therapy required to prevent death.

Every AE was recorded regardless of the possibility of being vaccine related. For each occurred AE the time of onset, outcome, intensity and relationship to the vaccine was evaluated and documented in the case report form. All solicited local reactions following vaccination were considered causally related to the vaccination.

The relation to the vaccine was assessed with the guidance presented in table 4.

Table 4: *Grading of relationship of adverse events to the study vaccine*

Causality grading		Explanation
1	**No Relationship**	No temporal relationship to study product and alternate etiology (clinical state, environmental or other interventions); and does not follow known pattern of response to study product
2	**Unlikely**	Unlikely temporal relationship to study product and alternate etiology likely (clinical state, environmental or other interventions) and does not follow known typical or plausible pattern of response to study product.
3	**Possible**	Reasonable temporal relationship to study product; or event not readily produced by clinical state, environmental or other interventions; or similar pattern of response to that seen with other vaccines
4	**Probable**	Reasonable temporal relationship to study product; and event not readily produced by clinical state, environment, or other interventions or known pattern of response seen with other vaccines
5	**Definite**	Reasonable temporal relationship to study product; and event not readily produced by clinical state, environment, or other interventions; and known pattern of response seen with other vaccines

Solicited Adverse Events

Solicited AEs were collected daily from the vaccination day until day 7 after each vaccination and on day 14 after each vaccination. In the following are lists of solicited AEs and their severity:

Table 5: *List of solicited local AEs and their grading of severity (* Grade 1: 2.5 – 5 cm, Grade 2: 5.1-10 cm, Grade 3: >10 cm, Grade 4: Necrosis or exfoliative dermatitis)*[186]

Local	Intensity	Parameter
Pain at injection site	0	Absent
	1	Minor reaction to touch
	2	Moderate reaction to touch
	3	spontaneously painful
Swelling at injection site	*	Record greatest surface diameter in mm
Induration at injection site	*	Record greatest surface diameter in mm
Erythema at injection site	*	Record greatest surface diameter in mm
Contra-lateral reaction	*	Record greatest surface diameter in mm
Pruritus at injection site	0	Absent
	1	Easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities
	2	Sufficiently discomforting to interfere with normal everyday activities.
	3	Prevents normal, everyday activities.

Table 6: *List of solicited systemic AEs and their grading of severity* [186]

Systemic	Intensity	Parameter
Fever	0	Tympanic temperature < 38°C
	1	38< and <38.5 °C
	2	38.5< and <39 °C
	3	> 39 °C
Nausea/vomiting	0	Behavior as usual
	1	Nausea/vomiting easily tolerated
	2	Nausea/vomiting that interferes with normal activity
	3	Nausea/vomiting that prevents normal activity
Headache	0	Behavior as usual
	1	No effect on normal activity
	2	Interferes with normal activity
	3	Prevents normal activity
Fatigue	0	Behavior as usual
	1	Fatigue easily tolerated
	2	Fatigue that interferes with normal activity
	3	Fatigue that prevents normal activity
Myalgia	0	Behavior as usual
	1	No effect on normal activity
	2	Interferes with normal activity
	3	Prevents normal activity
Diarrhea	0	None
	1	With no dehydration
	2	With some dehydration
	3	With severe dehydration

Serious Adverse Event

A serious AE is defined as follows: “A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

- Results in death;
- Is life threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect”[185].

Abnormal Laboratory values

The toxicity scales used to define abnormal laboratory values can be found in the annex. They were modified from the “Vaccines Guidances - Guidance for Industry: Toxicity Grading Scale for Healthy Clinical Trial Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials”[186] published by the federal drug administration. Alteration was performed along a database with reference data of adult Gabonese subjects in order to adapt the toxicity scale to our study population. Abnormal laboratory findings that were considered to be clinically significant were staged and recorded as AEs.

2.3 Laboratory assessment

2.3.1 Immunological assays: Indirect ELISA

To assess the IgG concentration of each participant against GLURP, MSP3 and GMZ2, indirect enzyme-linked immunosorbent assay (ELISA) was performed as previously described[112,121–123]. ELISA is a commonly used quantitative immunoassay to assess the concentration of antigen-specific antibodies in serum.

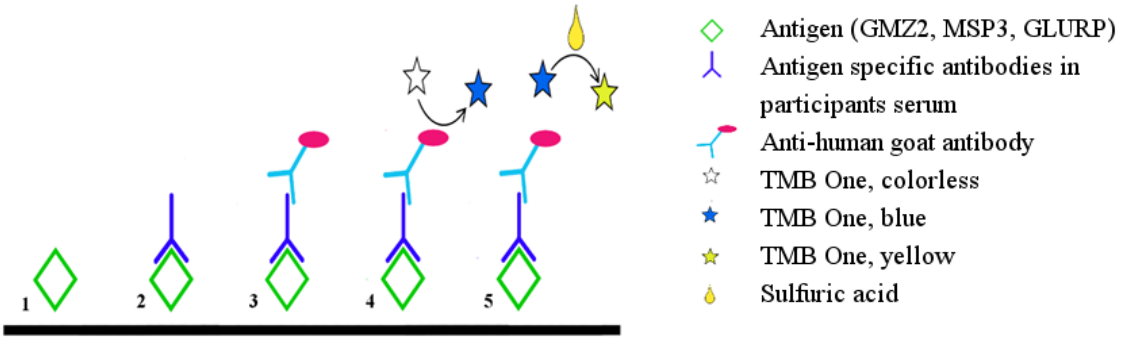


Figure 11: *ELISA. First, the antigen of interest is coated to a microtiter plate [1]. Afterwards, patient serum with the antibodies of interest is added, which bind the antigen, which in turn is coated on the plate [2]. Next, goat anti-human antibodies conjugated to a peroxidase are used. They bind the participants antibodies [3]. Then, a color solution is added, which react with the peroxidase conjugated to the goat IgG. The peroxidase channels a change of color [4]. The Tetramethylbenzidine One solution turns blue. To end the reaction, sulfuric acid is added, which itself turns the solution into yellow [5]. The intensity of the color change is directly proportional to the amount of specific serum IgG bound to the antigen on the plate and can be quantified by a plate reader.*

Blood preparations

For the analysis blood was drawn on day 0 and 84 directly centrifugated and the resultant participant plasma was stored in the refrigerator at -80 °C. Thus, it was possible to analyze all samples at the same time and under the same conditions. This took place at the end of the follow up period. The list and references of the reagents used are given in table 14 in the annex. Coating antigens were prepared by diluting GMZ2 (123µg/vial, Michael Theisen, Denmark) in 1ml H_2O

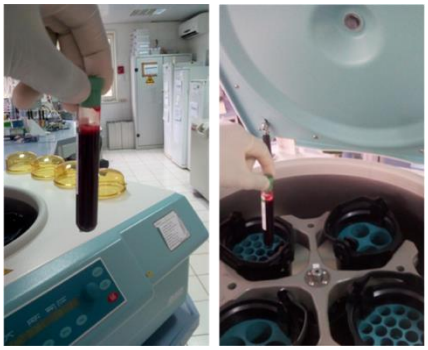


Figure 12: *Participants blood sample in the centrifuge*

and further diluted in Phosphate buffered saline (PBS) until a final concentration of 0.5µg/ml. MSP3 and GLURP at a concentration of 1µg/ml and 0.5µg/ml respectively.

The ELISA was performed in six steps as further described below:

Coating of microtiter plates

During this step 96 well microtiter plates were coated with 100µl per well of GMZ2, GLURP or MSP3 antigens. The plates were stored overnight in a fridge at 2 to 8°C to ensure sufficient coating.

Washing and blocking

The washing and blocking steps were performed the next morning. During this phase dilution buffer was discarded from the plates. In order to remove non-bound antigens, the plates were washed four times using a washing buffer. After the last wash cycle, the plates were gently inverted and tapped firmly on an absorbent paper to remove any remaining wash solution. Afterwards, every area on the plate, which was not fully coated with antigen, is blocked by a non-specific protein solution. Hereby, milk powder diluted in blocking buffer was used. This is done to prevent unspecific serum antibodies from binding on the microtiter plate and subsequently prevent them to interfere in the reading process later. The plates were then incubated with 150µl blocking buffer for one hour on a rocker platform at room temperature. This was followed by four subsequent washing procedures with washing buffer as described above.

Incubation with sample sera and standard IgG

As the next step, the plates were filled with positive-negative controls, standard serial dilution and participant's serum as specified below. This was done in order to allow binding of antigen specific IgG in participants-serum to the antigens coated on the plate.

As shown in figure 13, the first two columns of the microtiter plate were used for development of the standard IgG curve. Starting from a concentration of 30,000 ng/ml IgG, a serial dilution row was created with following concentrations: 15,000 ng/ml IgG, 7,500 ng/ml IgG, 3750 ng/ml IgG, 1.875 ng/ml IgG, 938 ng/ml IgG, 469 ng/ml IgG, and 235 ng/ml IgG. Further wells were used for the negative and positive controls. Pooled sera from semi-immune individuals of Lambaréné served as positive controls, whereas sera from malaria-naïve Europeans were used as the negative controls. Moreover, a few

wells were filled with dilution buffer, so that the standard IgG and the participant's serum photo absorbance values could be adjusted later with the values of the pure dilution buffer. Remaining wells were filled each with the participant's sera samples. For each sample six wells were used and filled with sera in decreasing concentrations. Two wells served as an identical pair; The first were filled with sera diluted with dilution buffer 1:2, the second pair diluted 1:4 and the third pair diluted 1:8. This is further illustrated in figure 13. As a next step the plates were incubated for two hours at room temperature, while the specific antibodies in the participant's serum bound to the antigen coated on the microtiter plate. The higher the concentration of specific IgG in the patient serum, the more binding to the antigens coated to the microtiter plate.

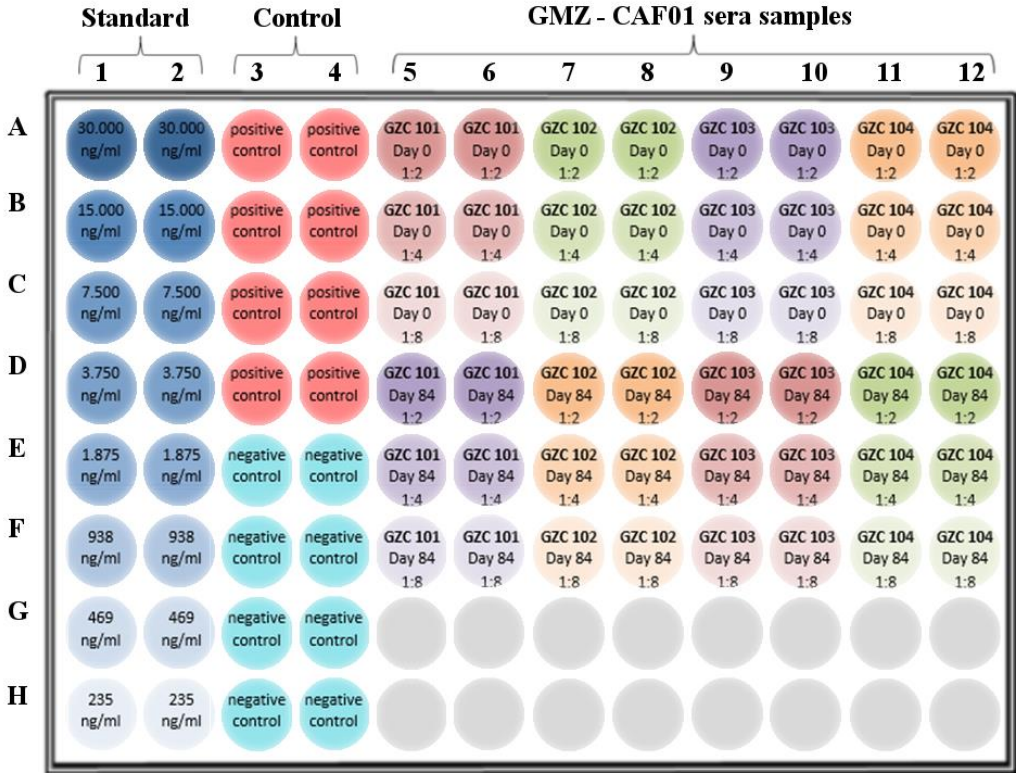


Figure 13: *Example microtiter plate*

Detection

Another four cycles of washing procedure followed the incubation. Next, 100µl of peroxidase-conjugated goat anti-human IgG diluted 1:65,000 with dilution buffer was added in each well. These anti-human antibodies bound on the antigen specific participant serum antibodies and the standard IgG during one hour of further incubation. Subsequently, antigen-antibody-antibody-peroxidase complexes were created.

Coloration

Now the plates were rewashed and 100µl substrate solution Tetramethylbenzidine was added. While the plates were light protected incubated for 20 minutes, the colorless Tetramethylbenzidine-One solution was oxidized by the peroxidase conjugated on the anti-human goat antibodies. Subsequently, it changed its light absorption at 450nm and turned into blue. The reaction was stopped after the 20 minutes by adding 100µl of sulfuric acid (100µl 0.2M H_2SO_4). This ensured that every plate had exactly 20 minutes of incubation. Due to the adding of the sulfuric acid, the solution changed the color from blue to yellow (620 nm).

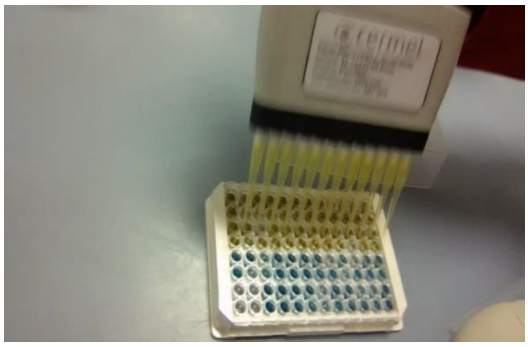


Figure 14: *Adding of sulfuric acid and the subsequent change of color*

Reading

This light absorbance was read by a plate reader at 450 nm, 620 nm respectively. The light absorption density is directly proportional to the amount of peroxidase enzymes in each well, therefore proportional to the number of anti-human goat antibodies and finally to the amount of specific participant serum antibodies against the antigen coated on each plate.

Figure 15: *Plate reader with an example ELISA-plate*

Calculation of the standard IgG Curve

For the calculation of the IgG standard curve, the mean between the two columns of the serial dilution row was calculated at each concentration. For example $(A_1+A_2)/2$ is calculated for the mean absorption at the concentration at 30µg/ml. The same was done for the decreasing concentrations in row B to H. Afterwards the mean absorption of all blanc wells containing dilution buffer was generated. In the example in figure 13 it was the mean values of G5-G12 and H5-H12. This value was *inter alia* used to adjust the means of the serial dilution row. Now, each adjusted light absorption value of the standard IgG dilution row could be matched with known concentrations of the standard

IgG. With these paired values a standard curve was calculated: $y = a + b * x$. Hereby, x represents the natural logarithm of the antibody concentration and y the logit of normalized light absorption values.

Calculation of the amount of specific antibodies in participants sera sample

In comparison with the standard IgG curve the antibody titer of each patient's serum sample could now be calculated. For this procedure, the mean light absorption values of each concentration of the samples were calculated. As an example, the serum sample of the participant with the number 101 on day 0 is taken: $(A_5+A_6)/2$, $(B_5+B_6)/2$ and $(C_5+C_6)/2$. The mean light absorption values were adjusted with the mean value of the blanc wells. Afterwards the interim amount of antibodies was calculated by using the standard IgG curve. The solution was picked, which was closest to the OD1. Next, the solution was adjusted according to their concentration in the beginning. In the example GZC101 on day 0, the interim amount of specific antibodies of row A was multiplied by two, since it was diluted 1:2. Row B followed the same procedure but was multiplicated with four and C was multiplicated with eight.

2.3.2 Diagnosis of *Schistosoma haematobium* infection

(*S. haematobium*) was detected microscopically by determination of eggs in urine. The method for identification consisted of two steps. In the first step 10ml of participants freshly collected urine were passed through a 12µm millipore Whatman filter placed on a filter holder. After the step of urine filtration, the filter was removed from the filter holder and placed on a transparent slide. The slide was then examined for the presence of *S. haematobium* eggs by microscope using the 10x objective and a closed iris condenser for sufficient contrast. The eggs were identified by size, shape and spine (figure 16).

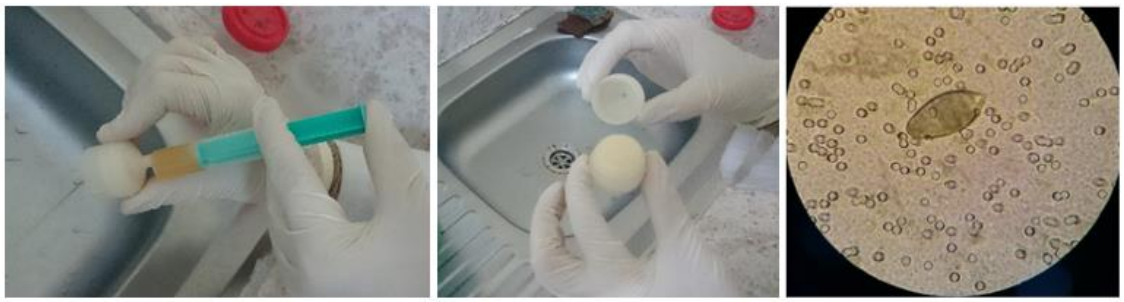


Figure 16: *Passing urine through the filter (left), unattached Whatman filter (middle), egg of S. haematobium under 10x magnification*

2.3.3 Detection of *Ancylostomatidae, Strongyloides stercoralis*

The presence of *Ancylostomatidae, Strongyloides stercoralis* were assessed by detection of larvae in the stool using the copro culture[187]. This method consisted of several steps: First, using a spatula, a small quantity of stools was transferred on a piece of aluminum foil. Afterwards, a sieve was pressed on the sample, so that the fecal material passed through. Meanwhile, a microscopy slide was wrapped in absorbent tissue and placed in a petri dish. A good quantity of the sieved stool was removed with a spatula and transferred on the tissue. Afterwards, sufficient sterile water was added the petri dish, such that the tissue was moisten but the stool samples was not covered (figure 17). Then, the petri dish was incubated for 7 days at 25°C (+-3°C).

Figure 17: *Sieved fecal sample on aluminum and applying stool on the tissue (left), incubated petri dish (right)*

For the filtration process, a 12µm Millipore Whatman filter was placed on the support of a syringe filter holder and afterwards the holder was reassembled. Then, the syringe was filled with 10ml of water of the incubated petri dish and attached to the filter holder to pass the water through the filter. The filter was placed on a slide. The entire filter was examined for larvae of *Ancylostomatidae,* and *Strongyloides stercoralis* by a phase-contrast microscope using the 10x objective and a closed iris condenser for sufficient contrast. Species differentiation was done by shape and size (figure 18).

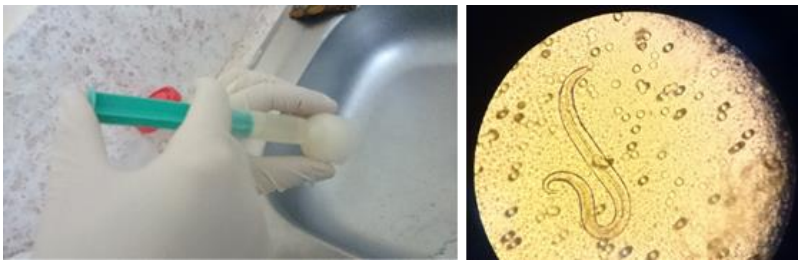


Figure 18: *Pressing incubated water through the Whatman filter (left), Ancylostomatidae under 10x magnification (right)*

2.3.3 Detection of *A. lumbricoides* and *T. trichiura*

Infection with *A. lumbricoides* and *T. trichiura* was determined by detection of eggs using the Kato Katz method[188]. For this procedure cellophane strips were soaked in 3% malachite green glycerol solution for at least 24 hours. The excess glycerol was drained before usage. A small amount of stool was transferred to a piece of aluminum foil followed by a screen, which was pressed on the sample for sieving.

Figure 19: *Items for analysis (left), sieved stool sample on aluminum (right)*

Afterwards, a template of 41.7mg (Vestergaard Frandsen SA, Aarhus, Denmark) was placed on a microscopy slide and a flat sided applicator stick was used to fill the hole of the template with the sieved sample. Subsequently, the template was carefully removed and the remaining stool on the slide was covered with the cellophane strip. Next, the slide was inverted, placed on absorbent paper, and pressed. Consequently, the sample was pushed against the cellophane and spread evenly. For each sample two slides were prepared. The prepared slides were read within 30 minutes and re-read after 24 hours. The entire slide was examined systematically by microscopy for intestinal helminths eggs using the 10x objective and a closed iris condenser for sufficient contrast. Not encapsulated eggs in the size of 50-65µm*20-30µm with clearly protruding plugs were taken for *T. trichiura*, whereas non-encapsulated, non-plugged, non-spined eggs in the size of 45-70µm * 35-45µm with a rough shell containing rough granules were diagnosed as *A. lumbricoides.*

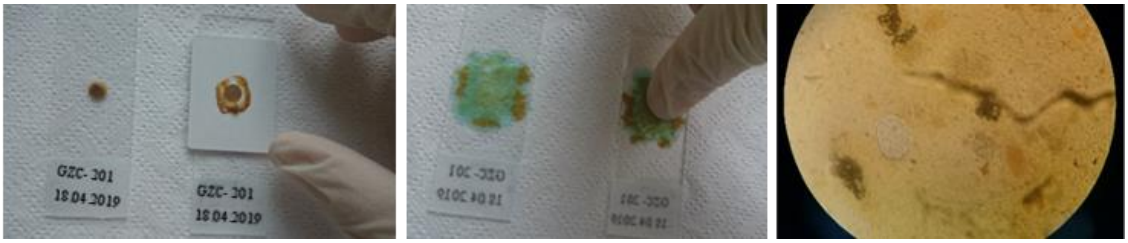


Figure 20: *Slides with template and stool sample (left), stool covered with cellophane strip (middle), egg of A. lumbricoides under 10x magnification*

2.3.4 Detection of *P. falciparum*: Thick blood smear

For detection of a *P. falciparum* infection a TBS was done applying the Lambaréné method[189]. Ten microliters of blood were taken and spread on a 10x18 mm large rectangle on a microscope slide. Next, the slide was dried and stained with a 20% Giemsa solution. After rinsing the slide, it was air-dried and read using a light-optical microscope. Parasites were counted at 1000x magnification. The parasitemia per microliter was calculated using the counted parasites, the counted high power field (HPF) and the microscope factor: $\frac{Parasites}{\mu L} = \frac{N_{parasites}}{N_{HPF}} * Microscope\ Factor.$

Figure 21: *Well-earned pause of an exhausted student after hours of malaria slide reading*

The microscope factor represents the number of high-power fields, which are needed to be read to examine 1 µl of blood. It is specific for each microscope and can be measured or calculated.

2.3.5 Detection of *Loa loa* and *Mansonella filarial* infections:

To assess filarial infection status of the study participants 10 ml of blood were collected in ethylenediaminetetraacetic acid tubes during the morning on day 0 and 84. Detection of microfilaria was performed following a modified Knott's technique[190]. The RBCs within the participants blood were lysed and the blood centrifugated. Afterwards the sediment was transferred to a slide and examined for motile microfilariae by microscope.

2.3.6 Hematology

For hematology examination, an ABX Pentra 60 (Horiba, Kyoto, Japan) was used to assess the number of erythrocytes, the HGB concentration, the hematocrit, the platelet count, and the total and differential leukocyte count.

2.3.7 Biochemistry

Biochemistry parameters including creatinine, AST, ALAT were measured by Cobas Mira Plus (Roche, Basel, Schweiz).

2.4 Data management:

The data generated by the study were documented on paper in the patient record form which represents the source document. Afterwards the information was transmitted to the case report form (CRP) and transcribed into a validated database (OpenClinica) following the Clinical Data Interchange Standards Consortium guidelines. In order to avoid transferring errors the data was filled in by two independent professionals. Regular monitoring was performed in accordance to GCP. All CRFs were verified by the clinical trial monitor with use of the source documents.

2.5 Statistical analysis

All data were analyzed with non-parametric methods using R (Version 3.5.2) and the packages 'tidyverse', 'readxl', 'ggpubr', 'ggimage', 'reshape2', 'magrittr', 'dunn.test', 'MASS', 'FSA', 'pracma', and 'car'. A Wilcoxon signed-rank test was applied to compare continuous variables such as anti-body titers between two groups (un-paired test) or timepoints (e.g. day 0 against day 84, helminths infected groups against non-infected, paired tests). If more than two groups were involved (x-fold ratio of antibody increase among study groups), a Kruskal-Wallis test was used. For hierarchical testing within the groups, the Wilcoxon signed-rank test was applied for pairwise comparisons in case the overall test was significant. The correlation between to continuous variables was analyzed applying the Spearman's correlation test (e.g. Number of antibodies against number of AE). The level of significance was set at a two-tailed type I error alpha <5%.

2.6 Sample size justification

An appropriate Sample size was determined with regards to the primary tolerability immunogenicity endpoints. The further analysis of the relationship between the immunogenicity and the tolerability profile of the malaria candidate vaccine was explorative. The same applies for the analysis of the impact of helminths infection on the candidate vaccine immunogenicity.

2.7 Ethics

Ethical approval was given in February 2015 by the "Comité National d'Ethique de la Recherche" in Libreville. It is a legally mandated institution by the Gabonese ministry of health. The study was conducted in compliance with the study protocol, the GCP, the Good laboratory practice (GLP) and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). The informed consent was obtained before any study procedure took place according to the current edition of the Declaration of Helsinki (2013). All data concerning the identification of a participant were treated as confidential. All analyses were done on pseudonymized data. The safety of participants was ensured by a local safety monitor and a scientific monitoring committee. If a participant fell ill or a helminth infection was detected, the participant was treated according the national guidelines. The trial is registered with the Pan-African Clinical Trials Registry, trial number PACTR201503001038304.

3 Results

3.1 Study flow and baseline characteristics of the study population

A total of 91 subjects were screened for study eligibility of whom 16 persons were not eligibly, mostly due to medical reasons, 6 declined to participate and 69 met all the inclusion criteria and none of the exclusion criteria. Of the 69 eligible individuals 50 were enrolled in the study, randomly assigned to one of the study groups and received their first vaccine dose (figure 22). Before the completion of the vaccination regimen three study participants moved out of the study area. Two of them left after the first vaccination on day 7 (Group A) and 14 (Group D) respectively and the third left on day 30 (Group A), which is after the second vaccination.

The age of the study participants ranged from 18.1 to 37.4 as displayed in table 7. Demographic characteristics of the study groups such as age, body mass index (BMI), HGB level, the amount of white blood cells, thrombocytes and the baseline IgG titers for GMZ, MSP3 and GLURP were similar between groups. Helminth infection was present in 21 (42%) of the study participants. *S. haematobium* was diagnosed in 15 individuals (30%) and intestinal helminths affected 10 individuals (20%). Poly-infection with *S. haematobium* and intestinal helminths accounted for 5 (10%) subjects. Distribution of helminth infection among the study group is depicted in table 7.

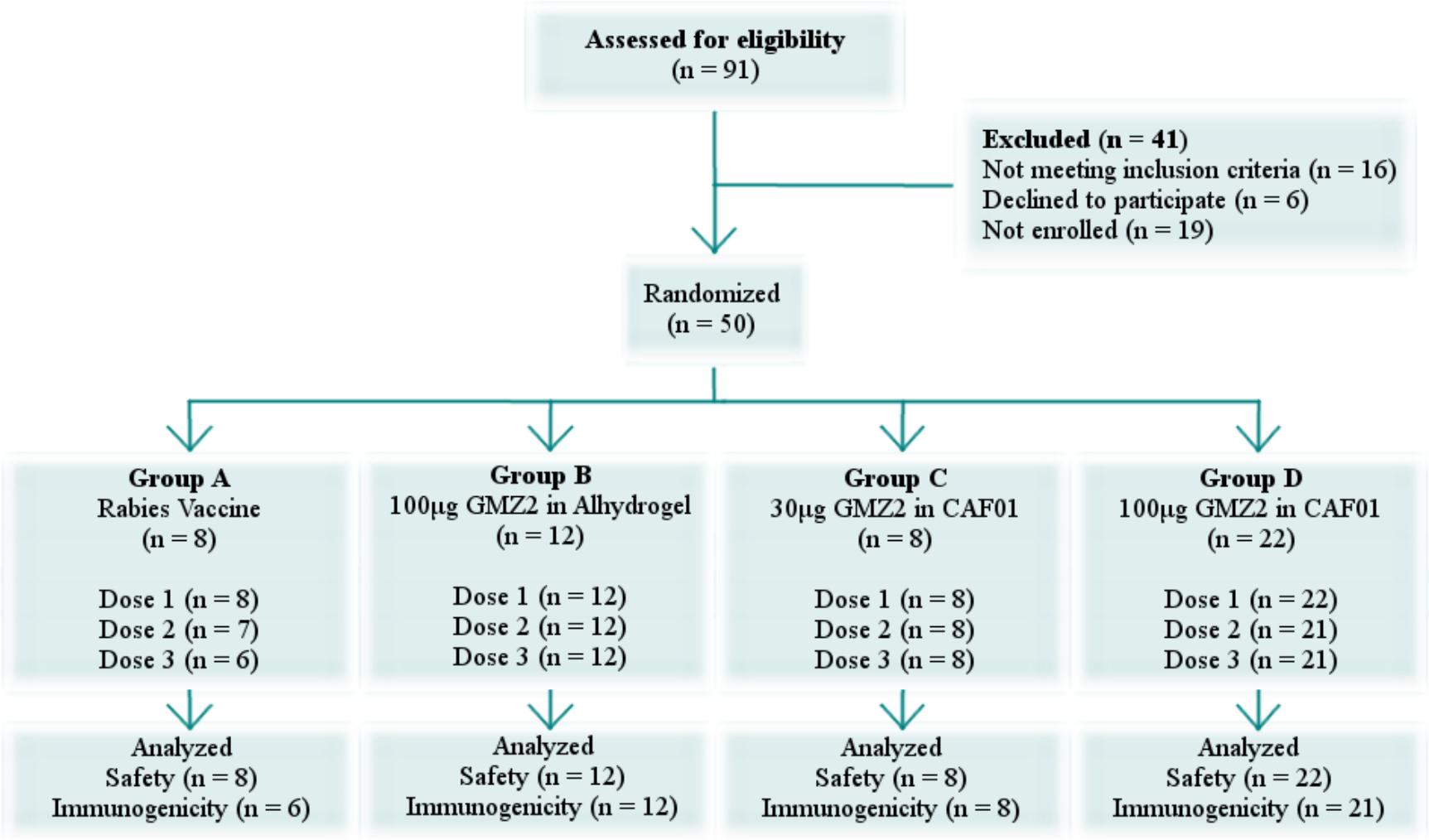


Figure 22: *Study flow diagram*

Table 7: *Baseline characteristics of vaccine groups with *median, (minimal – maximum value), #helminths infections at day 0 and/or day 84 (absolute numbers of infected subjects and infected percent)*

	Total (n=50)	**Group A** (n=8)	**Group B** (n=12)	**Group C** (n=8)	**Group D** (n=22)
Age *	22.7	22.8	24.4	22.4	22
in years	(18.1-37.4)	(21.8-35.5)	(19.2-32.2)	(20.2-35)	(18.1-37.4)
BMI*	22	22.5	22.1	21.7	22.5
in kg/m^2	(16.7-29.7)	(16.7-25.3)	(18.8-29.7)	(19.1-23.1)	(18.8-25.6)
HGB *	13.8	14	14.5	14	13.6
in g/dl	(11.1-16.3)	(12-15.3)	(12.7-16.2)	(11.1-15.7)	(11.7-16.3)
White blood cells*	5.2	5.7	5.1	5.2	5.1
in cells/µl	(2.7-10.1)	(4.7-8.6)	(2.7-10.1)	(3.8-9.7)	(3.1-9.1)
Thrombocytes *	195.5	204.5	192	151.5	190
in cells/µl	(91-343)	(144-258)	(162-317)	(91-267)	(98-343)
GMZ2 IgG*	1357.4	2025	1242.3	1240	1266.8
ng/ml	(413-5973)	(1053 – 2493)	(670-3916)	(572-5134)	(413-5973)
MSP3 IgG*	1161.3	1630.1	1373	1112.4	1075
ng/ml	(429-12929)	(547-2893)	(452-3987)	(429-12929)	(478-12514)
GLURP IgG*	1470.9	1252.5	1418.2	814.7	1616.5
ng/ml	(546-9162)	(657-3049)	(583-2582)	(546-4267)	(557-9162)
General helminth infection #	21 (42%)	3 (37.5%)	3 (25%)	4 (50%)	11 (50%)
Schistosomiasis #	15 (30%)	1 (12.5%)	2 (16.7%)	3 (37.5%)	9 (40.9%)
Intestinal helminths #	10 (20%)	2 (25%)	2 (16.7%)	1 (12.5%)	5 (22.7%)
Trichuris #	4 (8%)	0	1 (8.3%)	1 (12.5%)	2 (9.1%)
Ascaris #	1 (2%)	0	0	0	1 (4.6%)
Hookworm #	7 (14%)	2 (25%)	1 (8.3%)	0	4 (18.2%)
Other helminths infections #	3 (6%)	0	0	0	3 (13.6%)

3.2 Safety and tolerability

Regarding the analysis of safety and tolerability, the intention to treat population was analyzed. Thus, every participant was considered whether or whether not he completed the whole vaccination schedule. During the follow up period of the study from day 0 to day 84 no serious AE was recorded, and no participant had to be withdrawn concerning safety reasons. 221 AEs occurred in total, 196 being Grade 1, 25 Grade 2 and none Grade 3. 130 of those were at least possible related to the study vaccines (115 Grade 1, 15 Grade 2). The 15 related Grade 2 AEs were distributed on 13 participants and consisted of 14 times injection site pain and one episode of myalgia. They were all recorded in GMZ2-immunized participants (4 Group B, 5 Group C, 6 Group D). Two participants had no AE during the follow up period (1 Group A and 1 Group B).

3.2.1 Solicited local adverse events

The number of local AEs went from 27 (50%) to 34 (68.8%) and 26 (55.3%) after first, second and third vaccination respectively as shown in figure 23. 73 were Grade 1 and 14 were Grade 2. The most frequent AE was pain at injection side (71 Grade 1 and 14 Grade 2) and the only Grade 2 local AEs. It occurred after 58.6% of all vaccinations. One swelling and 1 pruritus at injection site (Grade 1, each) was observed. Indurations, erythema at injection site or contra-lateral reactions were not observed.

The rabies group showed lower AE rates (33.4% AE per dose (p.d.)) compared to the groups with GMZ2 formulations (60.4% p.d.), whereas the frequency among the GMZ2 groups where similar and not dose-dependent (Group B 51 % p.d., Group C, 96% p.d., Group D 56% p.d., figure 23).

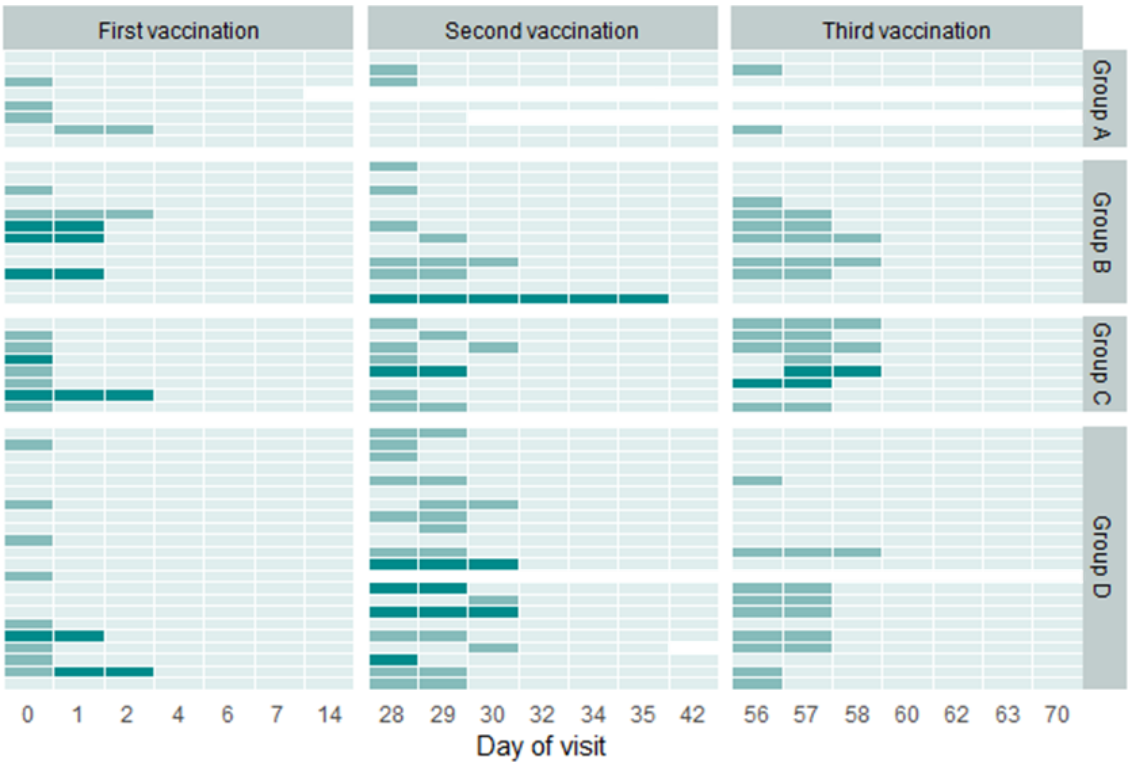


Figure 23: *Solicited local AEs recorded following vaccination. Each row represents one participant. Given is the Grade of the AE (highest intensity at each day of follow up) as shading (from light turquoise [no AE] to dark cyan [Grade 3]). Missed visits are indicated in white.*

3.2.2 Solicited systemic adverse events

A total number of 66 solicited systemic AEs were experienced by 31 participants. 38 of those were judged to be at least possible related to the study products. The amount of solicited related systemic AEs were 17 (24%), 10 (23%), and 11 (25.5%) following vaccination 1, 2 and 3 respectively. Three subjects developed a Grade 2 systemic solicited AE. One of them was judged to be possible related to the study vaccine. It occurred on day 61 (myalgia) in a subject vaccinated with 100µg GMZ2-CAF01. The frequency of AEs was similar among the groups as shown in table 8.

Table 8: *Rate of solicited systemic AEs (* average amount of AEs per person)*

Group	**Total** (n = 50)	**A** (n = 8)	**B** (n = 12)	**C** (n = 8)	**D** (n = 22)
Solicited systemic AE	66 (1.38*)	11 (1.37*)	18 (1.5*)	10 (1.25*)	27 (1.22*)
Related to study	38 (0.76*)	5 (0.63*)	14 (1.17*)	7 (0.88*)	12 (0.55*)
Diarrhea	14	2	4	3	5
Related to study	7	2	3	1	1
Fatigue	10	2	3	2	3
Related to study	10	2	3	2	3
Fever	4	1	2	0	1
Related to study	2	0	2	0	0
Headache	23	6	2	4	11
Related to study	10	1	2	3	4
Myalgia	4	0	1	0	3
Related to study	1	0	0	0	1
Nausea	11	0	6	1	4
Related to study	8	0	4	1	3

3.2.3 Unsolicited adverse events

A total of 68 unsolicited AEs was recorded. 60 of them were mild (Grade 1) and 8 were moderate (Grade 2). The number of unsolicited AEs were 18 (34%), 28 (39.6%), and 22 (34%) following vaccination 1, 2 and 3 respectively. The 5 Grade 1 unsolicited AEs, which were judged at least possible related to the study vaccines, were two times pruritus (Group B, day 28 and Group D, day 64), loss of appetite (Group C, day 0), asthenia (Group B, day 0), and pyuria (Group A, day 28). They were equally distributed among the vaccination groups as depicted in table 9.

Table 9: *Number of unsolicited AEs recorded during the vaccination period (*AE per person, **at least possibly related AEs)*

	Total		**Group A**		**Group B**		**Group C**		**Group D**	
Grade	I	II	I	II	I	II	I	II	I	II
All AE	60	8	7	1	16	1	12	2	25	4
(rate*)	(1.2)	(0.16)	(0.88)	(0.13)	(1.33)	(0.08)	(1.50)	(0.25)	(1.14)	(0.18)
Related AE**	5		1		2		1		1	
(rate*)	(0.10)		(0.13)		(0.17)		(0.13)		(0.05)	

3.2.4 Laboratory measurements

Abnormal values were distributed equally among the groups as shown in table 11. Their severity graded from 1 to 3. No Grade 4 value occurred. The only severe laboratory values (Grade 3) were low thrombocytes and low neutrophils, which occurred in all vaccination groups. No abnormal laboratory value was judged to be clinically significant. The values for creatinine, leucocytes, lymphocytes, and eosinophils stayed within the reference limits. The figures 24 to 26 show boxplots of laboratory parameters over all study visits and study groups.

Table 10: *Number of volunteers with abnormal laboratory findings by parameter and grade. The rate of events recorded per individual is indicated into brackets. Abbreviations: HGB: Hemoglobin, AST: Aspartate-Aminotransferase, ALT: Alanine-Aminotransferase, Neu: Neutrophils, Tho: Thrombocytes*

	Grade	**Total** (n=50)	**Group A** (n=8)	**Group B** (n=12)	**Group C** (n=8)	**Group D** (n=22)
HGB	1	14 (28%)		1 (8%)	7 (88%)	6 (28%)
	2	1 (2%)				1 (5%)
NEU	1	34 (68%)	3 (38%)	8 (67%)	7 (88%)	16 (73%)
	2	22 (44%)	1 (13%)	7 (58%)	4 (50%)	10 (45%)
	3	11 (22%)	1 (13%)	3 (25%)	2 (25%)	5 (23%)
THO	1	10 (20%)	1 (13%)	2 (17%)	6 (75%)	1 (5%)
	2	8 (16%)	1 (13%)		5 (63%)	2 (9%)
	3	5 (10%)	1 (13%)	1 (8%)	1 (13%)	2 (9%)
ALT	1	5 (10%)	2 (25%)			3 (14%)
	2	2 (4%)		1 (8%)	1 (13%)	
AST	1	5 (10%)	1 13%)	1 (8%)	1 (13%)	2 (9%)
	2	2 (4%)		2 (17%)		

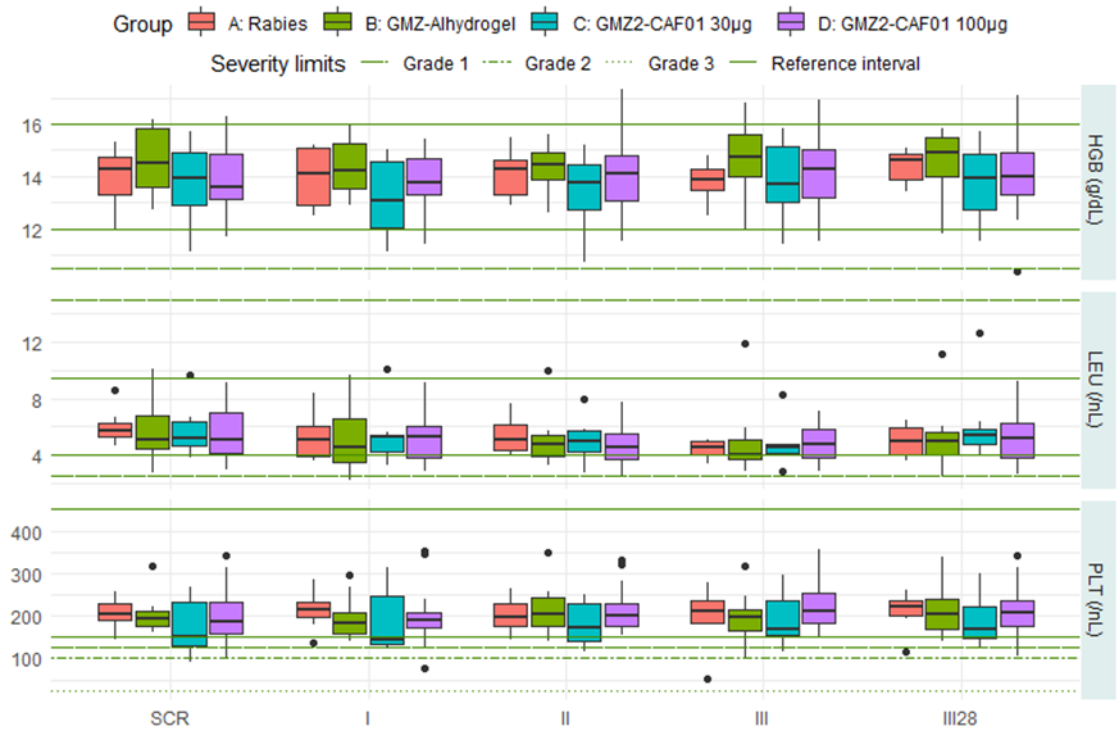


Figure 24 *Boxplots of HGB, leukocytes and thrombocytes for all volunteers at all visits. Dots represent outliers*

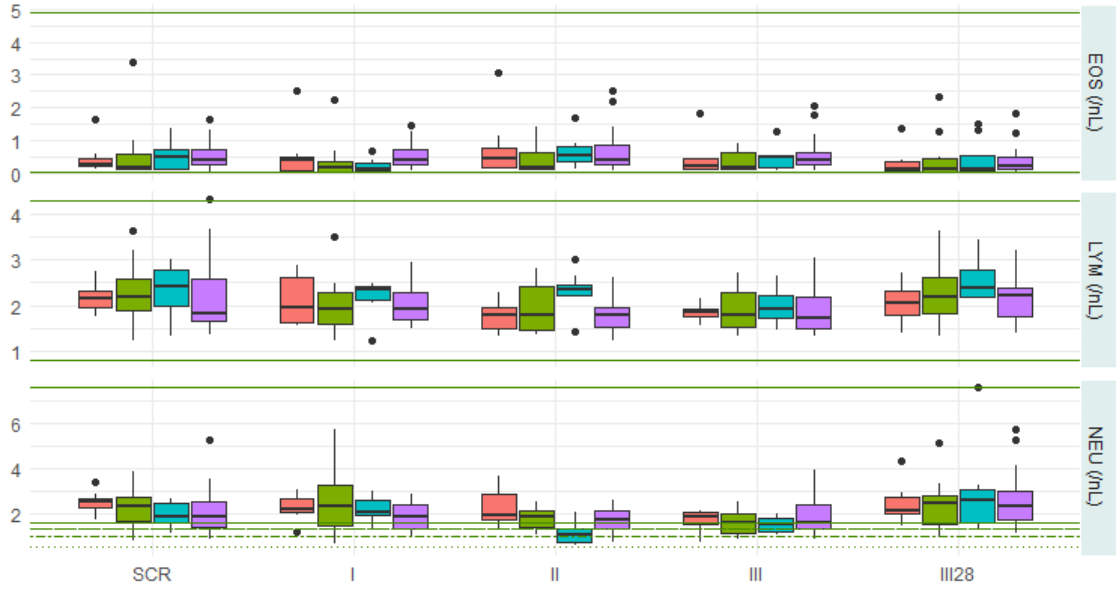


Figure 25: *Boxplots of eosinophils, lymphocytes and neutrophils for all volunteers at all visits. Dots represent outliers.*

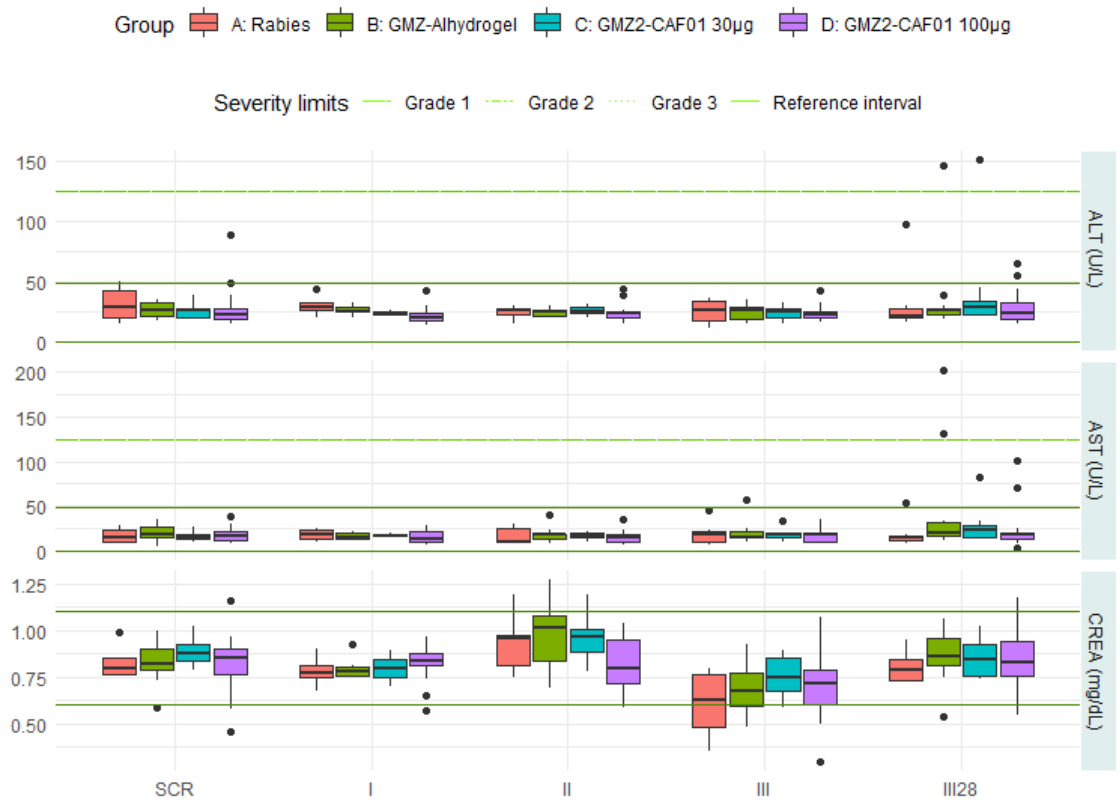


Figure 26: *Boxplots of ALT, AST and creatinine for all volunteers at all visits. Dots represent outliers.*

3.3 Immunogenicity

For the analysis of the immunogenicity the according-to-protocol study population was analyzed. Therefore, three participants, who did not complete their vaccination schedule were excluded from further analysis.

3.3.1 GMZ2 induced immunity

To determine the vaccine induced immunity, total anti-GMZ2 IgG as well as IgG against vaccine antigen subunits MSP3 and GLURP were measured before the first vaccine administration and on day 84. Of note: baseline level of IgG to GMZ2, MSP3, and GLURP were similar between the 4 different study groups (table 7 and figure 27), whereas the interindividual variability within the groups was rather pronounced. This variance converged strongly after the vaccinations on day 84 (figure 27). Vaccination with GMZ2 vaccine led to a significant increase of GMZ2, GLURP, and MSP3 total IgG in subjects who received GMZ2-Alhydrogel, 30ug GMZ2-CAF01, or 100ug GMZ2-CAF01. In contrast, the IgG titers against GMZ2, GLURP, and MSP3 of the subjects who received the comparator vaccine did not differ significantly when tested by Wilcoxon tests (figure 27). Moreover, a comparison of the fold increase of the measured antibody response against GMZ2 and GLURP IgG underlined the differences among the study groups as significant (Kruskal Wallis, figure 28). A further exploratory analysis showed a significant difference between the rabies vaccine and the GMZ2 formulations (pooled in one group regardless of the vaccine adjuvant and dosage). However, no difference was observed within the different GMZ2 vaccine formulations or dosages (figure 29). Regarding the immunogenicity of MSP3, the hierarchical testing in figure 28 was done exploratively, since the Kruskal Wallis was non-significant in the previous group testing (figure 27).

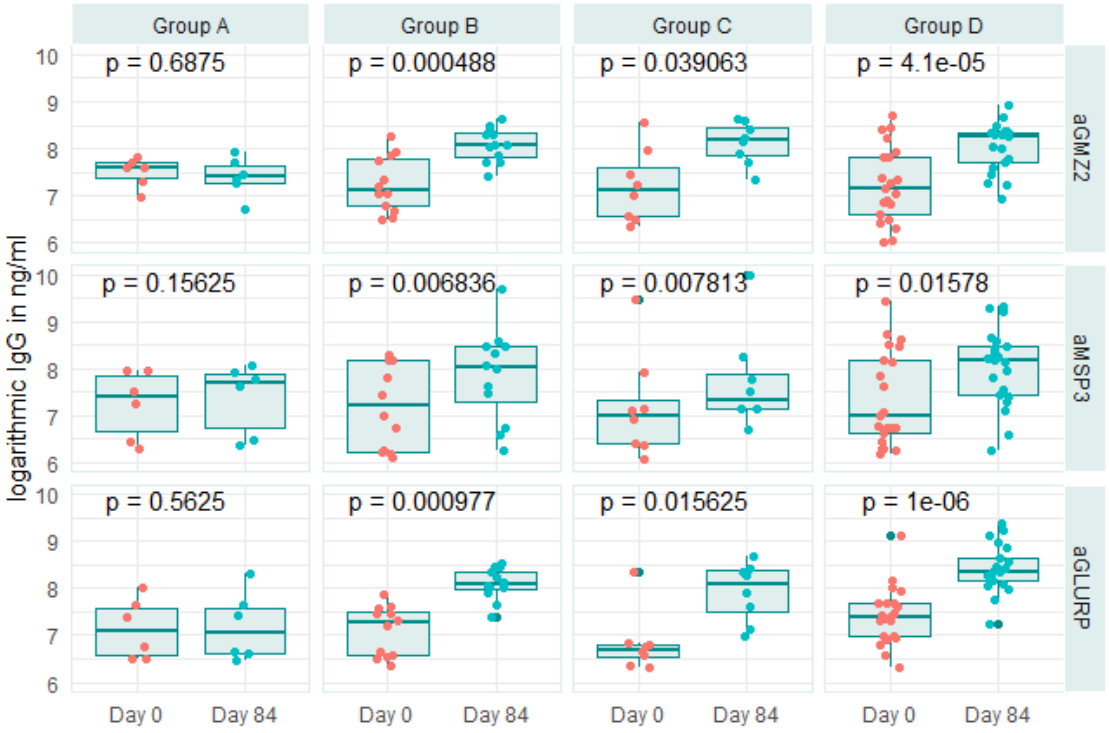


Figure 27: *Pairwise comparison of the level of IgG against GMZ2, MSP3 and GLURP at day 0 against day 84 with the Wilcoxon test.*

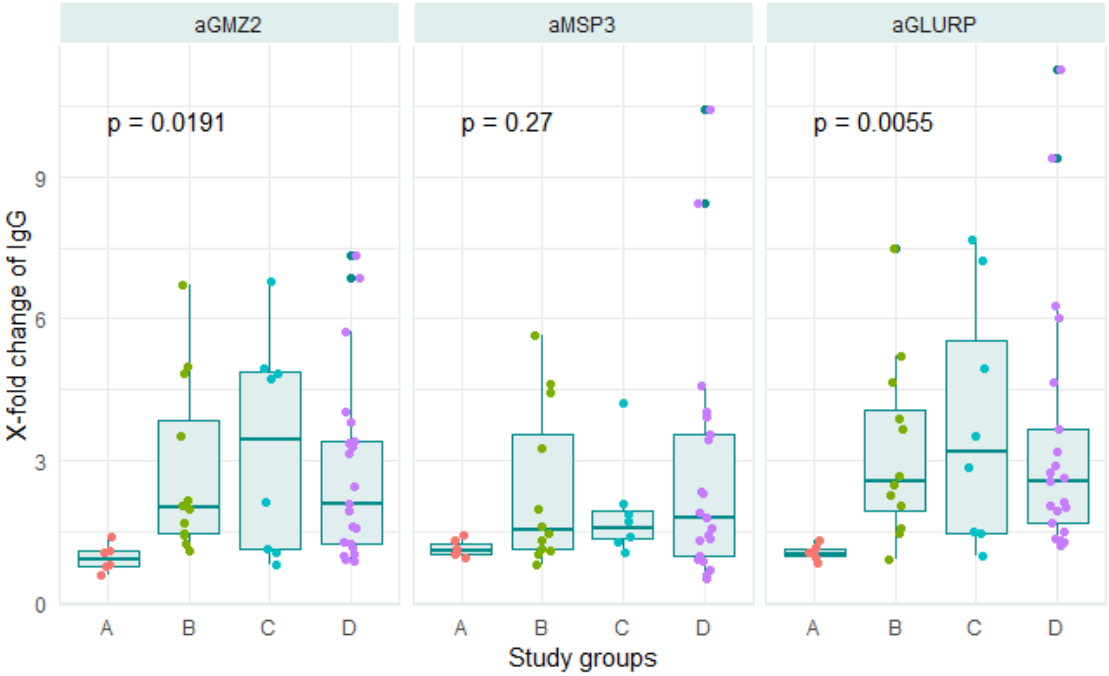


Figure 28: *Comparison of the x-fold change of IgG against GMZ2, GLURP and MSP3 among the different study groups using the Kruskal-Wallis test*

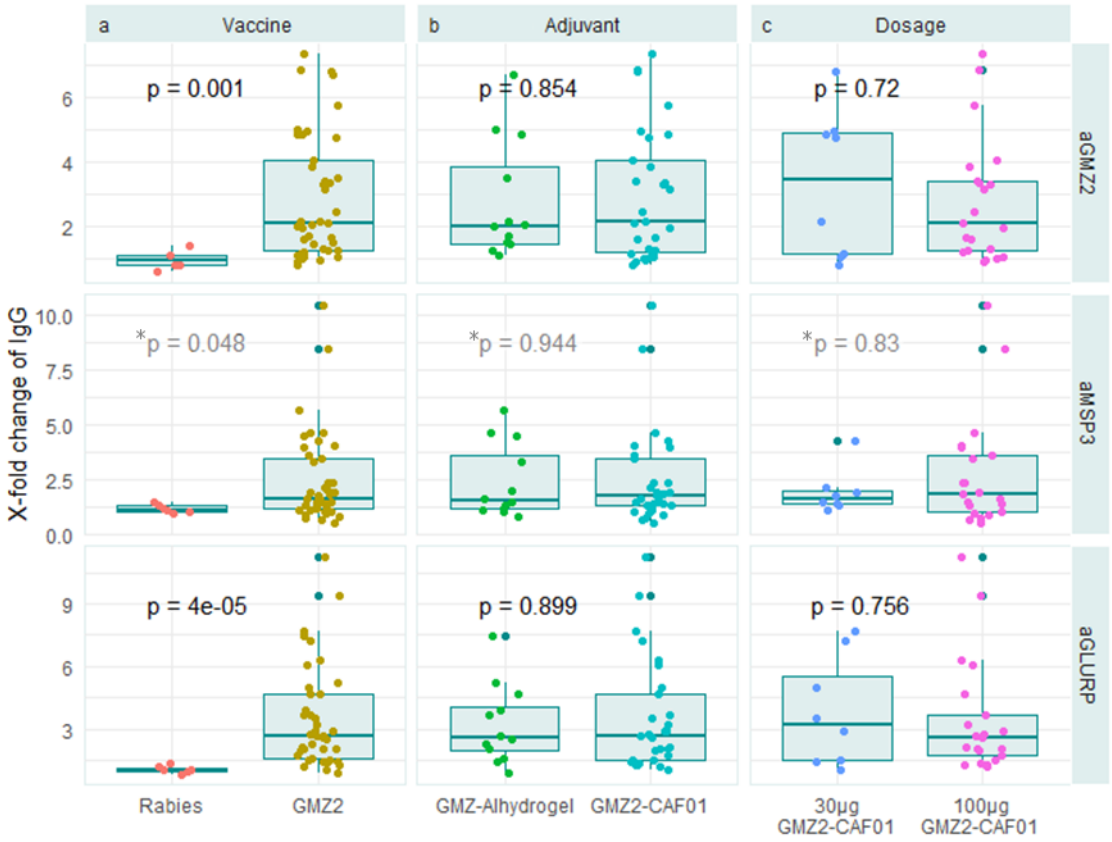


Figure 29: *Pairwise comparison of different study groups. The difference is analyzed between a) GMZ2 and the control vaccine rabies: All subject who received GMZ2 were grouped together, regardless of adjuvant and dose; b) Type of adjuvant used for GMZ2 formulation: All subjects who received GMZCAF01 were grouped together regardless of dose; c) The dose of GMZ2: Significance is assessed with the Wilcoxon test. P values regarding MSP3 are explorative and indicated with *.*

3.3.2 Effect of helminth infection on GMZ2 induced immunity

Baseline assessment indicate a prevalence of helminth infection ranging from 1 to 21 of infected participants depending of the species (table 7). In order to assess the effect of helminth infection on GMZ2 vaccine induced immunity, the fold increase of total IgG to GMZ2, GLURP and MSP3 was compared between helminths infected and uninfected subjects. Due to the low number of infected subjects, all vaccine recipient of the GMZ2 formulations were pooled together regardless of the vaccine, adjuvant and dosage. All subjects in the rabies group however were excluded from further analysis. As shown in figure 30, helminth infection resulted in a trend towards an increase to GMZ2 antibody in helminths infected subjects most pronounced in subjects infected by intestinal helminths. A comparable trend was observed for total IgG against GLURP and MSP3. The trend seems however to be weaker in subjects infected with *S. haematobium* compared to those with intestinal helminths.

Further analysis was computed with the objective to determine the effect of each intestinal helminth species on GMZ2 vaccine immunogenicity. Hence antibody level was compared between subjects infected and non-infected with *T. trichiura* or hookworm. The result of the analysis is shown in figure 31. It indicates that infection with either intestinal helminths species led to an increase of anti-GMZ2 IgG. Unfortunately, no analysis could be done for the infection with *A. lumbricoides* due to the small number of infected participants (infected: n=1). The same applies for *L. loa* infections.

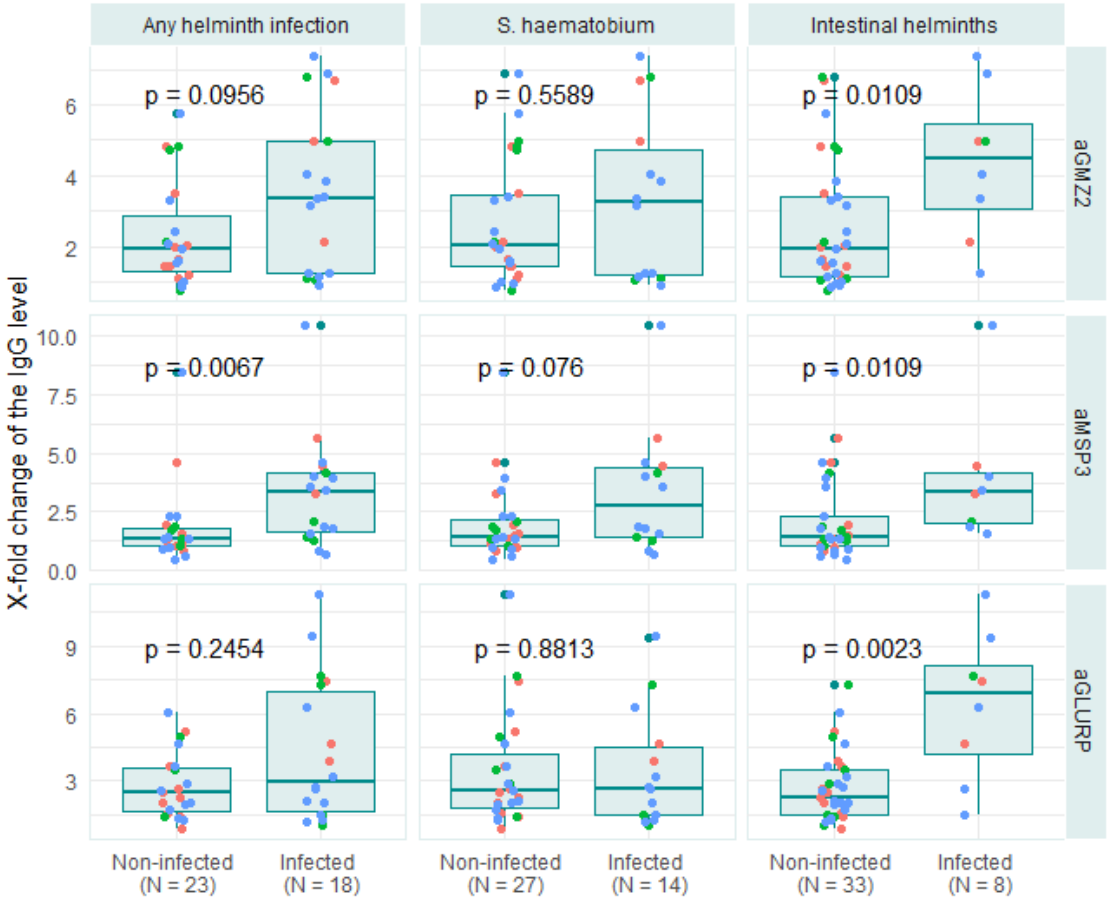


Figure 30: *Comparison of the x-fold change of IgG against GMZ2, MSP3 and GLURP between helminths infected and not infected participants with the Wilcoxon test. Number of observations are shown in brackets. In the first column participants with any helminth infection against non-infected are compared. The second and third columns show the s. haematobium and intestinal helminth infections respectively. The colors of the dots display the vaccination groups: Red = GMZ2-Alhydrogel, green = 30µg of GMZ2-CAF01, blue = 100µg of GMZ2-CAF01.*

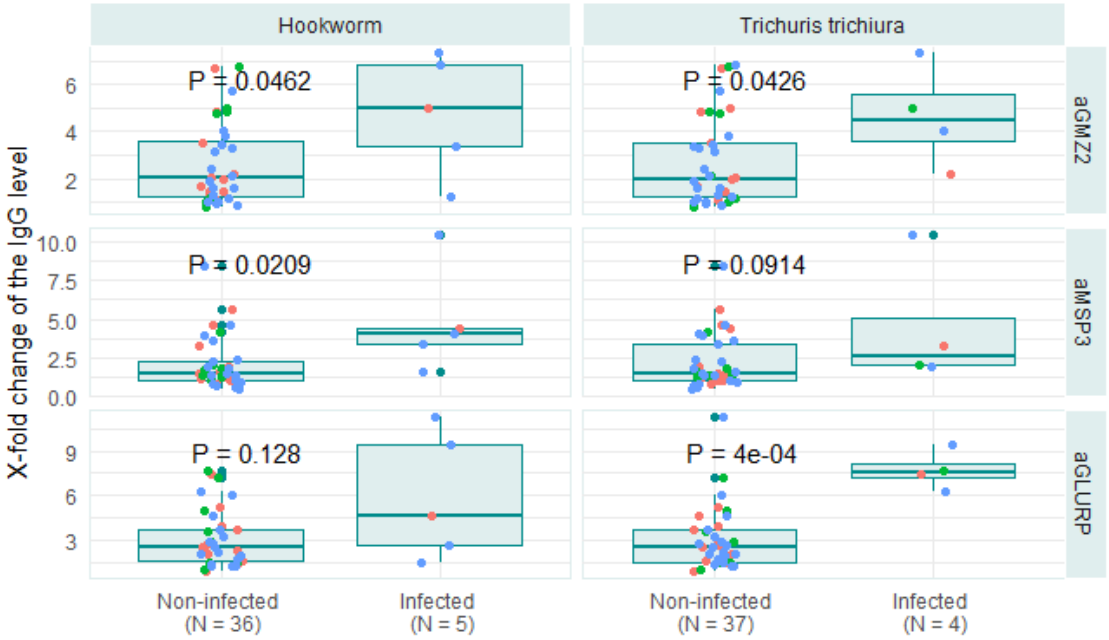


Figure 31: *Comparison of the x-fold change of IgG against GMZ2, MSP3 and GLURP between helminths infected and non-infected participants. Number of observations is shown in brackets. In the first and second column the Wilcoxon test is used to compare the infected with the non-infected groups of T. trichiura and hookworm respectively. The colors of the dots display the vaccination groups: Red = GMZ2-Alhydrogel, green = 30µg of GMZ2-CAF01, blue = 100µg of GMZ2-CAF01.*

3.4 Relationship between adverse events and the concentration of elicited antibodies

To assess the potential correlation between the vaccine-elicited inflammatory immune response and the occurrence of AEs an exploratory analysis was done. AEs were recorded from day 0 until day 84. Depending on the analysis different categories of AEs were created: Solely solicited AEs, every occurred AE, all AEs of severity Grade 2, abnormal laboratory events and severity of laboratory events. Analysis was restricted to participants receiving GMZ2 formulations and AE, which were at least possible related to the investigational product. Concerning the vaccine elicited immune response, the amount of antibodies against the different antigens at day 84 and the x-fold ratio of antibody increase were compared.

The main finding was that no strong correlation between the immune response and AEs could be established. Solely for the anti-GMZ titer at day 84 a weak relationship with the total amount of AEs ($r = 0.36$) was observed. The association is stronger, if the AEs are restricted to Grade 2 ($r = 0.43$) as shown in figure 32. Anti-MSP3-titers or anti-GLURP-titers did not show any association with AEs. The x-fold antibody increase did not show any correlation to the amount, severity or nature of AEs ($r = 12$ to $r = -22$).

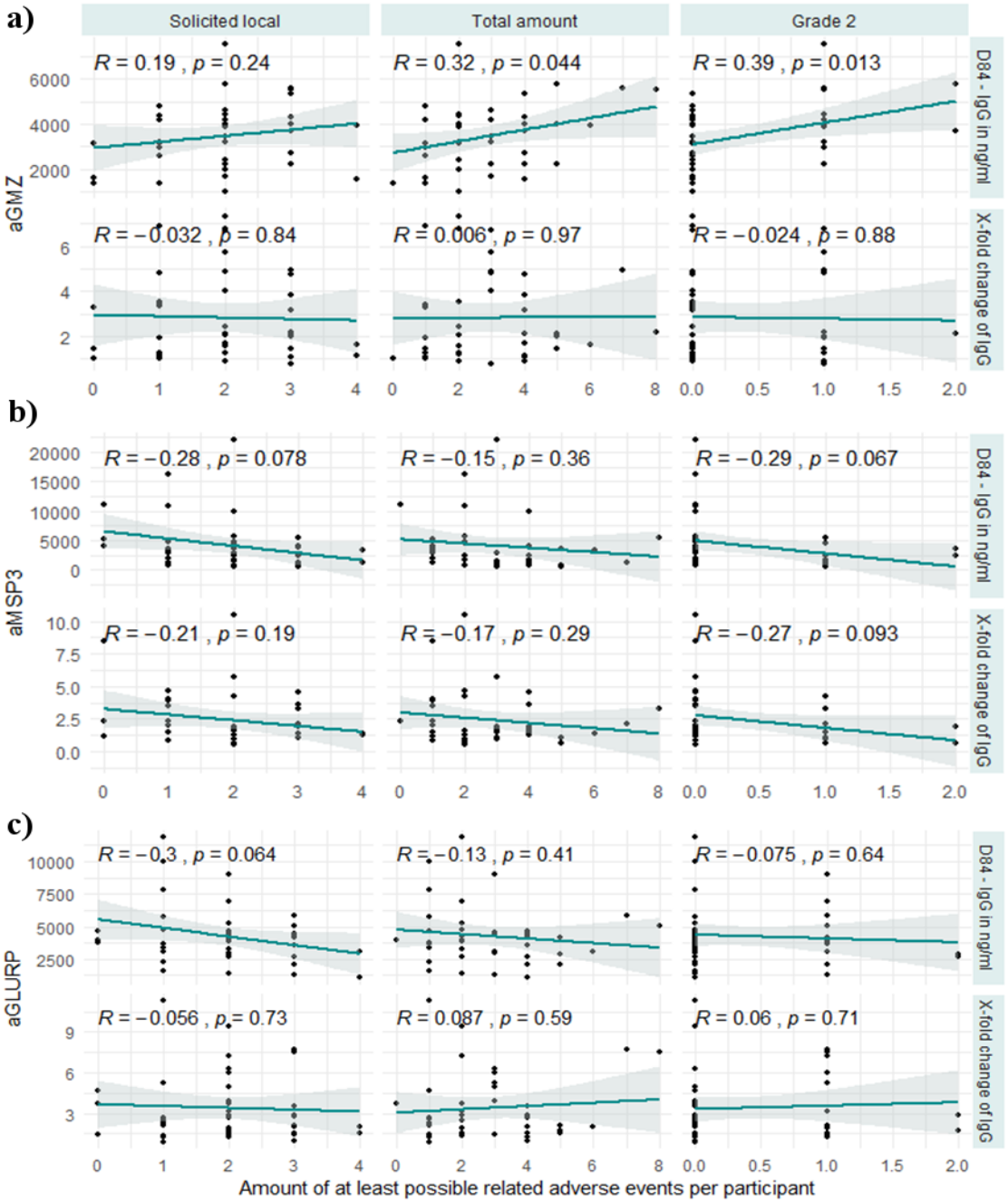


Figure 32: *Correlation between AEs and vaccine induced immunogenicity. The figure is divided by the different antigens: a) GMZ2, b) MSP3 and c) GLURP. The first row of each subplot contains the antibody titer against the antigen at day 84, whereas the second row displays x-fold ratio of antibody change. The first column shows the comparison against total amount of solicited local AEs per participant, whereas the second column displays all experienced AEs, which are at least possible related to the study vaccines and the third row is limited to at least possible related AEs of Grade 2.*

Secondly, it was assessed whether the baseline level of antibody titer may predict the occurrence of AEs. In order to address this question, the analysis was extended to vaccine specific antibodies at day 0 (analysis displayed in the annex: Figure 34 to 36). Though, no correlation between baseline IgG and occurrence of AEs was established either.

Thirdly, an analysis regarding abnormal laboratory values was performed. On a primary approach, total amount of abnormal laboratory values and their severity were evaluated in correlation against vaccine specific antibodies at day 84 and the x-fold ratio of IgG rise. The analysis did not show strong correlations (r = -0.036 to r = 0.14) (figure 33). On a second approach, the analysis was extended in order to establish a potential correlation between trends in laboratory values and vaccine specific antibodies. Baseline corrected area under the curve for each laboratory value was calculated and associated with the IgG titer at day 84 and x-fold change (analysis displayed in the annex: Figure 37). In this analysis likewise no clear relationship could be depicted. Noteworthy is a weak negative correlation between the dynamics of HGB and the IgG values.

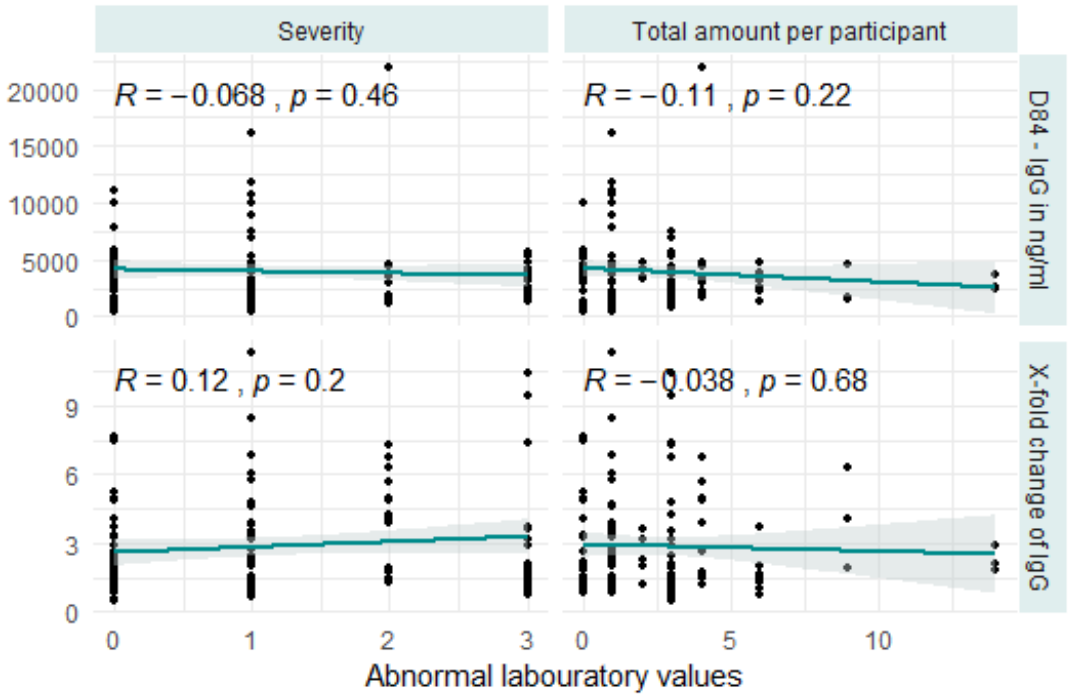


Figure 33: *Correlation between abnormal laboratory values and the vaccine induced Immunogenicity. The first row contains the antibody titer against GMZ2, MSP3 or GLURP at day 84. And the second row displays the x-fold ratio of the antibody increase. The first column shows the severity of abnormal laboratory values (Grade 1-3), whereas the second column*

displays the comparison against the total amount of experienced abnormal laboratory values events per participant.

In summary, no correlation between the elicited immune response and AEs could be established, except for a weak association between the experienced AEs and the antibody titer against GMZ2 at day 84.

4 Discussion

Despite being a disease, which is easy to diagnose and to treat, malaria continuously poses a high burden to mankind. It has a heavy impact on health indicators marked by a loss of approximately 55 million disability-adjusted life-years[191] and a mortality of around 435,000 (2017). Further, it constitutes a huge financial burden due to *inter alia* medical costs, reduced worker productivity and premature mortality. A country with falciparum transmission has a 1.3% lower economic growth rate[192].

As a result of global efforts, malaria incidence was reduced by 37% during the years 2010 to 2015[4]. The recent years, however, were marked by stagnation. Despite continuous global endeavors (US$ 3.1 billion spend in 2017[7]), no further progress has been achieved in the reduction of the disease burden[7]. Moreover, previous achievements are under continuous pressure by increasing threats. These are *inter alia* represented by emerging parasite resistance to anti-malarial medicines[193] and mosquito resistance to insecticides[7]. In order to accelerate and coordinate the efforts against malaria, the WHO designed a global strategy formulated in the "*Global Technical Strategy for Malaria 2016–2030".* Within the document the WHO defines a set of global goals to reach until 2030. These goals include reduction of malaria morbidity and mortality rates by at least 90%, elimination of malaria in at least 35 countries, and prevention of resurgence of malaria in all malaria-free countries[66]. To accomplish these objectives, new tools in the fight against malaria are needed. Of particularly value would be an anti-malaria vaccine. Vaccines are the most cost-effective intervention for public health[72]. They have been utilized in several previous eradication programs such as polio[73], smallpox[74], and measles[75]. Thus, the WHO has set the goal to develop a second generation malaria vaccine with protective efficacy of at least 75% over one year by 2030[193]. Currently, development of only one malaria vaccine has completed phase III: RTS,S (Mosquirix). It has received a positive scientific opinion from the European Medicine Agency[83] and is currently being administered to children in a selected number of countries as part of a phase IV implementation study.

Beside RTS,S, there are several other vaccine candidates. Among the BSV, the vaccine category of GMZ2, there are currently several candidates evaluated in clinical efficacy trials. AMA-1 is one of the most studied BSV antigens. It is a key protein of the

merozoites to invade RBC, but it is extensively polymorphic. By now, relevant efficacy (64%) was only shown against vaccine like strains with no significant overall reduction of malaria incidence[106]. MSP1 is a highly abundant surface protein, which is also essential for RBC invasion. Antibodies against MSP1 were protective in preclinical studies and associated with efficacy in clinical trials[194]. Yet, a recent phase IIb trial showed no protection despite of inducing a high magnitude of antibodies[103]. The combination of AMA1 and MSP1 administered in viral vectors showed little efficacy in a CHMI trial[195]. MSP3 induces high titers of cytophilic IgG1 and IgG3[196] and offered partial protection against malaria episodes[120]. Another vaccine candidate, Combination B, consists of MSP2, RESA and MSP1. It showed 62% reduction of parasite density, however the protection was strain specific and no overall clinical efficacy could be demonstrated[105].

GMZ2 is a BSV candidate, with an excellent safety and tolerability profile[102], and moderate efficacy. The first-in-man phase I trial in healthy malaria naive adults took place in Tübingen in 2006 to assess its safety, tolerability, and immunogenicity in humans. Since the study was successful[121] and the vaccine showed a good safety and tolerability, the next trial moved from malaria naïve participants to semi-immune adults to assess whether the observed results can be reproduced in a population with lifelong exposure to malaria. Thus, a phase I trial was conducted in Gabon in 2007. It confirmed the good safety of the vaccine candidate and showed, that the pre-existing immune response to vaccine antigens can be boosted[122]. Following this trial, the clinical development proceeded and a phase I trial in the target population of healthy African children was conducted in 2008. This vaccine trial indicated that the vaccine was well tolerated in children from 1-5 years of age[123]. In order to determine the vaccine efficacy under natural exposure to the parasite, a large phase IIb multicenter study was conducted in 2010 in four countries (Gabon, Burkina Faso, Ghana, and Uganda). A total of 1849 children were included and followed up for one year. The results of this trial confirmed the good tolerability of the vaccine. However, it showed a vaccine efficacy of only 13.6% (95% CI: 3.6%, 23%), which was statistically significant but not high enough to warrant further development[124]. Interestingly, the capability of inducing efficacy at distinct study sites suggested that the vaccine was pan-reactive in the sense that it may not be limited by strain specific immunogenicity. Moreover, a significant relationship between the

immunogenicity and vaccine efficacy could be established, which indicates that a more immunogenic GMZ2 formulation capable of eliciting higher antibody titers could significantly increase the overall vaccine efficacy.

While GMZ2 has already been safely tested in clinical trials, it was always adjuvanted with aluminum hydroxide, which has an excellent safety record dating back almost a century ago[128]. However, the downsides of this adjuvant are the rather poor immunogenic features compared to more recently developed adjuvants. Several other adjuvants have been proposed as an alternative to aluminum hydroxide. Among them is the CAF01 adjuvant. It is an adjuvant with potent immune- enhancing properties on humoral and cellular responses[197]. It has been successfully assessed with other vaccine candidates against diseases such as TBC[135], HIV[136,] and malaria[131]. In preclinical GMZ2 studies CAF01 vaccine formulations showed superiority over aluminum adjuvanted ones[108,131]. Therefore, the question was posed whether GMZ-CAF01 may improve the GMZ2 vaccine immunogenicity without altering the safety of the vaccine.

To answer the question, this phase I vaccination trial including a CHMI was conducted. CHMI is a method to evaluate *inter alia* malaria vaccine candidates in early proof of concept clinical studies, which has recently standardized by using inoculation of cryopreserved sporozoites[198]. Standardized CHMI can be used to conduct highly reproducible studies in malaria-free and malaria-endemic setting to obtain first efficacy data within small phase I studies without the direct necessity of cost-intense phase II clinical studies in malaria endemic settings. Ahead of the trial already 73 volunteers had been infected with CHMI in controlled clinical trials by intravenous injection of PfSPZ[199]. They were well tolerated and safe. The total number of volunteers, who underwent different kinds of CHMI, is in the four-figure range[200]. The results of the CHMI of this trial are discussed elsewhere[172]. This thesis covers the immunological and tolerability aspects of the vaccination phase of this clinical trial.

4.1 Study population

The clinical development of a new malaria vaccine normally starts with phase I trials in young healthy adults within a malaria naïve population. This is due to several considerations: First, safety aspects: A first in human trial poses less risks for the study subjects if conducted in a healthy naïve population, compared to the risks for a population already burdened with infection. Moreover, adverse events are easier and clearer recognized. The difference in safety conditions in distinct endemic vs non-endemic situations is illustrated by the vaccine candidate Na-ASP-2 against hookworms. First it was used and proved to be well tolerated in the USA[201], but a subsequent trial in Brazil had to be halted due to generalized urticarial reactions[202]. Secondly, immunological aspects: The impact of the vaccine on the immune system is easier evaluated in absence of preformed IgG against malaria antigens. Preexisting immunological changes due to former infections with *p. falciparum* may otherwise conceal subtle effects.

When the malaria vaccine candidate's tolerability profile is positively validated in a malaria naïve study population and it elicits a robust immunological response, the clinical development proceeds to tolerability and immunological evaluation in a malaria endemic situation. There, the tolerability profile can be further analyzed under endemic conditions. Moreover, the question can be answered whether preexisting levels of immunogenicity can by further boosted by immunization and efficacy data can be obtained. Nevertheless, the paradigm that large phase III trials under natural exposure is the first stage when efficacy data can be obtained is questioned by the increased use of CHMI, which is likely a good surrogate for efficacy under natural exposure[203,204].

Not only regarding safety, but also from an immunological point of view, the transit from a non-endemic to an endemic study population can be challenging. This is demonstrated by several oral vaccines[139–141], and especially by the PfSPZ vaccine: Reaching 80-100% vaccine efficacy in European or US populations[80,205], the efficacy dropped to 30-50% in Mali[206]. Further factors impairing vaccine efficacy and immunogenicity in malaria endemic settings are outlined in section 1.3.1.

While GMZ2-Alhydrogel followed this path of development[102(p2)], the combination of GMZ2-CAF01 started the clinical development directly in a malaria endemic setting. GMZ2 itself had been extensively clinically evaluated in combination with Alhydrogel[102] and CAF01 had shown a good safety and tolerability profile in combination with other

vaccines[135,136]. Thus, with regards to the safety, it was reasonable to conduct the trial directly in a malaria endemic setting.

In terms of immunogenicity GMZ2 had already proven the ability to elicit functional vaccine specific antibodies[123(p2)]. In the current study it could not only be evaluated, whether GMZ2-CAF01 elicits a robust IgG response, but also whether it is able to boost preexisting titers against vaccine specific antigens. And further, whether this boosting effect is superior to GMZ2-Alhydrogel. Additionally, insights form previous GMZ2 trials indicate, that immunological data obtained during trials with semi-immune adults may be translated to trials in the target population (1-5 years old children)[122(p2),123(p2)].

With an inoculation rate of about fifty infective bites per person per year, little seasonal changes[178,179] and an extensive record of clinical trials Lambaréné offered ideal conditions conducting the first in human trial in an malaria endemic setting. We chose fifty young healthy men as participants with a long-term residence over ten years in the area of Lambaréné to ensure a sufficient history of malaria transmission. The average baseline anti-body titer against the different vaccine antigens was 1309 ng/ml, therefore an adequate exposure can be assumed. Other baseline characteristics such as age, BMI, laboratory values (WBC, PLT), and helminths infections were similarly distributed among study groups. The level of baseline IgG among the study groups as well as the baseline characteristics were similar.

Participants did not benefit directly from participating in this study. However, to compensate for the time investments by participating in this trial, participants received expense allowance. Moreover, the information they gained about their general health status during study procedures may have a potential indirect benefit and a physician was available for them twenty-four hours a day during the whole study period. Furthermore, participants, who did not yet receive the rabies vaccine, were offered the vaccination at the end of the study period.

4.2 Safety and tolerability

Primary aim of this study was to assess the safety and tolerability of GMZ2 adjuvanted with CAF01 in semi-immune adults.

We hypothesized that GMZ2 adjuvanted with Alhydrogel would show a good tolerability as shown in previous trials[121–124]. Even though no previous data was available regarding the new formulation of GMZ2 adjuvanted with CAF01, we postulated an equally good tolerability of the new GMZ2-CAF01 vaccine formulation, based on the tolerability profile of other CAF01-adjuvanted vaccines.

In this study no serious AE and no Grade 3 AE occurred. A total of 221 AEs was recorded of which 130 were judged to be related to the investigational medicinal product. This difference is common for a vaccination trial and can be explained by the fact, that symptoms from common disease are reported during the study period, too. To distinguish between vaccine caused AEs and other AEs so called solicited AE were implemented in addition to the need to assess the causality of any AE. Solicited AEs are known to be related to vaccinations. Within the solicited AEs further differentiation was done between local and systemic AE, which are listed in table 5 and 6 of the methods section. All local solicited AEs were judged to be related to the study vaccine. In contrast, the relationship of the systemic solicited AEs was assessed individually by the recording study physician on a casual grading scale ranking from 1 (no relationship) to 5 (definite relationship) (table 4 method section). The same method applies to non-solicited AEs. A solicited systemic AE is still probable to be at least possible related to the study vaccine 58% (38 out of 66, tables 8 result section), whereas the unsolicited AEs were judged to be related in only 8.3% of the cases (table 9 result section).

Regarding the tolerability of the study group vaccinated with GMZ2-Alhydrogel, data obtained from previous trials did not raise any safety concerns. The most reported AE was pain at injection side, as it is to be expected from a subunit protein-based vaccine. The majority of AEs were of mild to moderate severity. A few serious AEs were recorded in these trials, however there were all judged not to be related to the study vaccine.

Regarding GMZCAF01, it was the first time the vaccine-adjuvant combination was evaluated. Therefore, no direct comparisons to former studies can be done. Nevertheless, CAF01 was assessed with other vaccine candidates against diseases such as TBC[135],

HIV[136,] and malaria[131]. Similar to the results in the current study, CAF01 had shown a good tolerability with focus on local AEs such as pain at injection side[135,207] or injection side movement impairment[135]. Systemic AEs were observed rarely [135,136,207].

4.2.1 Solicited local adverse events

The distribution of the solicited local AEs was quite similar throughout the first, second and third vaccine administration with no significant increase towards the last vaccination. This was observed in previous GMZ2-Alhydrogel trials, too. The majority (97%) of recorded AEs was pain at injection site, of which solely a minority (16%) reached Grade 2 severity ranking. Moreover, pain at injection site was the only Grade 2 local AE.

A similar trend was shown during GMZ2 precursor studies. Particularly throughout the phase II trial conducted by Sirima et al. pain at the injection site occurred with a similar frequency[124]. This is also observed from other vaccine trials where CAF01 was used as an adjuvant[135,136,207]. Moreover, a comparison of the results of the current study with data obtained from a phase I vaccine trial of GMZ2 was made. It was conducted by Mordmüller et al. in 2007 in semi-immune adults in Lambaréné and presented similarity in term of study design. As in the current trial, in the 2007 study the tolerability profile was characterized by occurrence of AEs, which were mainly mild to moderate with no Grade 3 AEs recorded. However, the pattern of local AEs was different. Mordmüller et al. observed higher frequencies of induration, erythema, pruritus, edema and local heat[122]. One possible reason for the difference in tolerability outcome between our trial and Mordmüller et al. is the route of vaccine administration. In the 2007 trial the vaccine was administered by sub-cutaneous injection[122], while in the current study it was injected via the intramuscular (i.m.) route. Using a muscle as a depot for the vaccine lead to less local reaction, because subcutaneous fat tissue is more sensitive and more likely to cause local irritations[208]. This is supported by the tolerability results of the two subsequent GMZ2 trials phase Ib[123] (2008) and IIb[124] (2010). While moving forward to the target population (children), the vaccine application form was changed from subcutaneous to i.m. This led to a substantial decrease in study vaccine related AEs per participant[121–124].

Regarding the distribution over the study groups, there is a slight difference with an average of 33.4% AE p.d. in the rabies group compared to 60.4% AE p.d. in the group of subjects vaccinated with GMZ2. The variation within the different GMZ2 formulation

is negligible. Neither the change of the adjuvant nor the different vaccination doses seem to influence the good tolerability of GMZ2.

The findings confirm the general good tolerability of protein-based subunit vaccines, which do not contain live components and are considered as very safe[209]. Previous studies with the single antigens GLURP and MSP3 showed a slightly different profile of solicited local AEs. Regarding MSP3, Hermsen et al. found a higher frequency of pain at injection site (55% p.d.), erythema (100% p.d.) and indurations (100% p.d.) upon vaccination with 100µg GLURP-Alhydrogel[210]. Similar in the case of GLURP, Sirima et al. (2009) observed an increased occurrence of pain at infection site (60% p.d.), swelling (64% p.d.), and induration (91% p.d.) after immunization with 30µg MSP3-LSP[196]. Moreover, their participants experienced more severe AE, namely swellings (Grade 2: 22% p.d., Grade 3: 42% p.d.) and indurations (Grade 2: 33.3% p.d., Grade 3: 58% p.d.)[23]. These differences may be once again explained by the variation in route of administration. Indeed, in contrast to the current study the vaccine was administered subcutaneously in these studies possibly resulting in a higher rate of local AEs. Further, in the case of MSP3 the choice of LSP as adjuvant may have contributed to the different study results.

If compared to RTS,S the leading malaria vaccine candidate, the tolerability profile is slightly different. RTS,S is also injected intramuscularly and induced fewer rates of pain at injection site (12.4% p.d.). But, it caused higher rates of erythema (3.1% p.d.) and swelling (9.6% p.d.)[82]. In contrast, the current study showed higher rates of pain at injection site (55% p.d.) but fewer other forms of solicited local AE: swelling (2% p.d.) and no erythema. During the large trials with RTS,S Grade 3 local AEs were reported[82]. However, they occurred in low frequencies, therefore our trial was not powered to detect rare, potentially more severe, AE.

The tolerability profile of vaccines against Hepatitis B, which are also adjuvanted with Alhydrogel and are an example of very commonly used subunit vaccines, is comparable to our results. Frequently observed are mild local AE: pain at injection site (2-29% p.d.), erythema (3% p.d.) and swelling (3% p.d.)[211]. Only pain at injection site occurred in higher frequency during our study: Grade 1 in 55% p.d., and Grade 2 in 35% p.d. of vaccinations.

4.2.2 Solicited systemic adverse events

Only 66 solicited systemic AEs were recorded during the study of which 38 were judged to be at least possibly related to the investigational medicinal product. In terms of intensity most AEs were mild and only 1 was considered moderate (day 61, myalgia, group D). With regard to the type of AE, headache accounted for most of the reported AE (7.1% p.d.), followed by fatigue (6.3% p.d.), diarrhea and nausea (4% p.d. each), fever (1.6% p.d.), and myalgia (0.8% p.d.) (AE rates p. d., at least possibly related to study vaccine, only GMZ2-formulations considered). In contrast to the pattern of local AE, no difference was observed between the rabies and the GMZ2 formulations (table 8).

In contrast to our results, the former GMZ2 phase I study conducted by Mordmüller et al. showed higher rates of solicited systemic AE: Headache 21.7% p.d., fatigue 18.3% p.d., diarrhea 15% p.d., nausea 13.3% p.d., and myalgia 11.7% p.d.. Interestingly, they did not report any episode of fever. Nevertheless, if compared to our overall rates of solicited systemic AE, the variation in observed AEs remains: While our participants experienced a solicited systemic AE after 43.7% of all vaccinations, Mordmüller et al. observed a rate of 80%. For the sake of comparability only systematic AEs were taken in account, which were also considered to be solicited in our study: Fever, fatigue, headache, myalgia, nausea, and diarrhea. Considering every solicited AE reported by Mordmüller et al (fever, contralateral reaction, fatigue, drowsiness, malaise, headache, joint pain, myalgia, loss of appetite, nausea, vomiting, diarrhea, and tachycardia) the rate of AEs increases to 145% AE p.d. [122].

Other difficulties occur while comparing our results to the data obtained from the GMZ2 phase II study. This is due to the difference in the type of solicited AEs recorded during both studies. In the phase II study, the list of solicited systemic AEs included “loss of appetite” (2.1% p.d.), “drowsiness” (0.9% p.d.), and “irritability” 0.6% p.d. Drowsiness may be substituted with fatigue for the sake of comparability with our results. Further one case of “absence of appetite” (2% p.d.) was observed but recorded as an unsolicited AE in our study. Regarding the corresponding AE, the phase II trial showed fewer frequencies of fever 1.34% p.d., drowsiness 0.15% p.d., and diarrhea 0.11% p.d. [124].

Parallel to this study Sirima et al. compared GMZ2-Alhydrogel to the rabies vaccine in a phase II study. Compared to our findings, the frequency of solicited systemic AEs in their comparator group was as well lower. They observed fever 1% p.d., drowsiness 0% p.d., and diarrhea 0.3% p.d. Our results show similar frequencies for fever <1% p.d., but higher frequencies for fatigue 8.3% p.d., and diarrhea 8.3% p.d. [124]. This indicates that the variation in tolerability pattern between Sirima et al. and our results may not be due to the different adjuvant (Alhydrogel vs CAF01), but due to more general factors such as the distinct study populations.

In a former phase I CAF01 trial, TBC vaccine Ag85B-ESAT-6 (H1) was adjuvanted with CAF01. Dissel et al. found a similar frequency of AE in comparison to our results. Fatigue and headache occurred slightly less with 1.5% p.d. and they additionally reported the occurrence of pruritus 0.5% p.d., and rash 0.5% p.d. [135]. These AEs were not considered as solicited in our study. Nevertheless, pruritus occurred two times and was recorded as an unsolicited AE (4% p.d.).

In the case of RTS,S, solicited systemic AEs similar to Sirima et al. were chosen: Loss of appetite 11.4% p.d., drowsiness 6.6% p.d., irritability 11.5% p.d., and temperature 31.1% p.d.. This tolerability profile is similar to our findings, apart from the high frequency of fever 31.1% p.d. and the occurrence of 2.5% p.d. Grade 3 temperature rise (cohort of children aged 5-17months at enrollment)[82]. The increased temperature may have contributed to the increased risk of febrile seizures, which was one of the major safety concerns raised in the context of RTS,S[84].

If compared to the commonly used subunit Hepatitis B vaccines, the most important solicited systemic AEs are temperature over 27.7°C (1-6% p.d.), headache (3% p.d.) and anaphylaxis (1.1 per 10^6 doses of vaccination). Apart from the anaphylaxis, which cannot be detected at such low frequencies in a phase I trial, also this tolerability is comparable to GMZ2CAF01.

4.2.3 Unsolicited adverse events

From the 68 unsolicited AEs a fraction of five was considered to be related to the study vaccination. The five Grade 1 AEs were two times pruritus, absence of appetite, asthenia, and pyuria. Absence of appetite, or asthenia are candidates for systemic solicited AE[82]. They were frequently seen in other vaccine trials. Nevertheless, in our case the frequency was too low to recommend the implementation of these AEs in the list of solicited AEs.

Pruritus on the other hand, as a non-local AE, is regarded as solicited in the CAF01 trial conducted by Dissel et al. There, it occurred after 0.5% of all vaccinations and in our case after 4%. Therefore, it may be recommended to implement it as solicited in future CAF01 trials.

In conclusion, GMZ2CA01 was well tolerated. Its tolerability profile is similar to the GMZ2-Alhydrogel profiles observed in previous trials and former CAF01 studies. Moreover, the AE pattern is comparable to other subunit vaccines as RTS,S and Hepatitis B. There are minor non-significant differences since most AEs were mild. Moderate AEs were rare, and no serious AE or Grade 3 AE occurred. A total of 130 possibly related AEs was reported during the study period. 85 were pain at the injection site of which 75 were Grade 1. Grade 1 indicates a minor reaction to touch after vaccination, the majority of those resolved within 48 or 72 hours. This would be a low price for a diminished malaria-risk. Nevertheless, this study was not powered enough to detect rare AEs and safety concerns may be raised during larger trials.

4.2.4 Abnormal laboratory values

In reference to the safety of our study participants, blood samples were routinely assessed for potential signs of organ damage. HGB was analyzed to detect anemia. Leucocytes, lymphocytes, neutrophils, and eosinophils were evaluated for abnormal findings regarding the immune system. ALT and AST were assessed for potential liver damage. Finally, creatinine was measured in case of kidney impairment.

The abnormal laboratory values were graded from 1 to 3 with the vast majority being Grade 1. The amount of abnormal laboratory value was equally distributed among study groups and none were considered to be clinically significant.

Elevated liver enzymes occurred seldomly, which could be due to *inter alia* non-specific liver irritations in the course of e.g. a virus infect or alcohol consumption. Neutropenia, which occurred slightly more frequent, is common after vaccinations and is mostly transient and benign. It can occur due to concurrent viral infections or co-medication (antiretrovirals and antibiotics)[212]. Thrombocytopenia may be explained by various reasons, among them an enlarged spleen in the course of a malaria infection. The same reason might apply for the few cases of lowered HGB.

Noteworthy is, that abnormal values were not only distributed equally among the study groups, but also among the various study visits including the screening visit before the first vaccination. Therefore, a correlation between the vaccinations and the abnormal laboratory values is not probable and the irregularities were more likely caused by common illnesses and random fluctuation.

4.3 Immunogenicity

4.3.1 GMZ2 induced immunogenicity

The second main objective of this study was to assess the antibody mediated immune response induced by GMZ2 adjuvanted by either aluminum or CAF01 in an adult population semi-immune to malaria. Our first hypothesis was that immunization with GMZ2 will lead to a significant increase in IgG to the vaccine antigens. Secondly, we postulated that adjuvating the GMZ2 antigen with CAF01 would lead to a significant increase of the vaccine induced immune response in comparison to the GMZ2-Alhydrogel formulation. Thirdly, we hypothesized, that a dose of 100µg GMZ2CAF01 would lead to a significant increase of the vaccine specific IgG in comparison to the dose of 30µg GMZ2CAF01.

Our study population has a lifelong history of recurrent malaria infections; thus, a certain baseline level of IgG against GMZ2, MSP3 and GLURP could be anticipated. And consequently, the IgG-titers at day 0 and day 84 were measured to analyze the rise and x-fold change of the antibodies instead of the absolute antibody concentration at day 84.

The first important finding was that the vaccination with GMZ2 led to a significant increase in specific IgG antibodies against MSP3, GMZ2 and GLURP - although more pronounced for the two later antigens.

Similar findings were presented in the previous GMZ2 trials where a significant increase of anti-GMZ2 and anti-GLURP IgG were reported and were more pronounced in comparison to the raise of anti-MSP3 IgG titer[121–124]. In the phase I and phase II GMZ2 trials conducted by Belárd et al. and by Sirima et al. respectively, the magnitude of the vaccine specific antibodies fold change was higher than in the current study. These differences can be accounted for by the difference in the study population. Indeed, in this study semi-immune adults were included, whereas in the phase II multi-center study and the phase I trial the study population was composed of children aged from 1 to 5. They have a shorter history of exposure to *P. falciparum* and their immune system presents different features. Repeated infections with *Plasmodium spp.* can lead to activation of immune regulatory mechanisms, which are probably more pronounced in semi immune adults than in children. This is further underlined by a study conducted in 2007 by Mordmüller et al.[122]. They evaluated the impact of GMZ2-Alhydrogel on semi-immune

Gabonese adults similar to our study design. There, a general less pronounced increase of vaccine specific IgG was shown in comparison to Bélard et al.[123] and Sirima et al.[124], which in turn corresponds with our results.

The second important finding was that no difference was observed in vaccine induced immune response between subjects vaccinated with GMZ2-CAF01 and subjects vaccinated with GMZ2-Alhydrogel. In contrast to aluminum hydroxide, CAF01 was selected as an adjuvant for its capacity to induce a strong and long-lasting memory cell mediated and humoral immune response. Our results indicate that, on the contrary to what was expected, changing the vaccine adjuvant from aluminum hydroxide to CAF01 did not lead to significant increase of the vaccine specific IgG response. Therefore, CAF01 will probably not improve GMZ2 vaccine efficacy in a significant manner in larger phase II or phase III trials. This finding contradicts the result of the pre-clinical study assessing the effect of different adjuvants on malaria vaccine. In that study, by comparison to Alhydrogel, CAF01 adjuvanted malaria vaccines were more immunogenic[131]. Similar findings were obtained from clinical trials on HIV[136,207] and TBC[135] trials, where CAF01 was used as an adjuvant and demonstrated potent immune enhancing properties. Regarding the trials for HIV and TBC, the variance in enhanced immune response may be explained by the different antigens used in the vaccine trials. Though vaccinated with the same adjuvant, the distinct vaccine antigens themselves still have different immunogenic properties. Moreover, our trial was the first to compare Alhydrogel and CAF01 head-to-head in a human trial.
In the case of the preclinical assessment, MSP1 was used to assess the immunogenic properties of CAF01 in combination with a malaria vaccine candidate. Apart from the general difficulties transferring pre-clinical successes into clinical trials, our study assessed different blood-stage antigens (MSP3 and GLURP). This may also explain the differences in outcome.

The third finding was, that a significant difference in the immune response induced by the two different formulations of 30ug and 100ug of GMZ2-CAF01 was not observed.

In reference to the induced immunogenicity and efficacy[124,172] it is unlikely, that these GMZ2 formulations can reach the requirements for a malaria vaccine defined by the

WHO. Still, it is proven that GMZ2 can elicit functional antibodies[118] and that the vaccine efficacy increases with higher immunogenicity[124]. In the current study no significant variations regarding immunogenicity and tolerability between 30µg and 100µg GMZ2-CAF01 occurred. Thus, a further dose escalation of either the vaccine antigens or the adjuvant may be needed for a sufficient immune response to the vaccine antigens. This is further supported by the better efficacy in high-responders in the phase IIb trial. The equally good tolerability regarding both dosages indicates that the dose escalations may be feasible in terms of tolerability.

Another straightforward approach may be to continue the search for a more suitable adjuvant for GMZ2. This is as well reflected in the development of the leading malaria vaccine candidate RTS,S, where it was not until several modifications of the delivery system (Alum to AS02/AS01), that RTS,S was capable to induce a significant level of protection[213]. Apart from aluminum salt and CAF01 formulations, there are different several adjuvants in clinical development such as Viral vectors (RNA or DNA based), MPL combinations (AS01, AS02, AS04), Montanide ISA-720, saponin-based (QS21) adjuvants and virosomes[126,214]. In a mouse model GMZ2 showed strongly improved immunogenic properties when attached to the surface of immunopotentiating reconstituted influenza virosomes[215,216]. If further pre-clinical testing confirms the adequacy of this approach, a phase I clinical trial will be necessary to assess safety and tolerability. Taking place in malaria naïve subjects, sera samples may be utilized to assess the quantity of elicited vaccine specific antibodies by ELISA. Functional assays such as opsonic phagocytosis of merozoites and ADCI could be used to evaluate in vitro parasite growths inhibition. If the resulting data indicates, that a robust and long-lived protection may be achievable, clinical research might proceed to develop an improved GMZ2 formulation. Nevertheless, the success of this approach is uncertain as the example of CAF01 demonstrates the difficulties of translating mouse models into human application in adjuvant research. Another example is the adjuvant GLA-LSQ, which showed superior immunogenicity against Alhydrogel in preclinical studies, however failed to improve the antibody response to PAMVAC in a phase I trial[95].

If an improved GMZ2 formulation can be obtained it may be combined with malaria vaccines targeting other stages of the parasite's life cycle[217]. A combination of GMZ2 with TBS or pre-erythrocytic vaccine candidates may not only increases the benefit on the subject level but also reducing the transmission on the population level, and diminish the risk of vaccine resistances[102,217].

Variation in magnitude of vaccine specific baseline IgG and at day 84

Interestingly, a large interindividual variance at day 0 was observed. This could be explained by exposure to different levels of malaria transmissions among the participants. The general environmental parameters impacting infection rates may be similar for our study subjects, since they were all recruited in the area of Lambaréné. However, the more individual factors might have varied: The household situation, proximity to freshwater puddles, and the usage of vector control measurements such as ITN.

Further, the large interindividual variance in baseline IgG converged strongly within day 84. This could due to predominant boosting of the immune response of the participants, which had a low baseline titer in contrast to less boosting of participants, who already had a high IgG titer from the beginning. This poses several questions: Until what limit can the IgG titer be boosted? Is there a ceiling effect? Do regulatory responses play a role? And can a BSV be efficient at medium IgG titers levels[218]?

4.3.2 Effect of helminths infection on GMZ2 induced immunity

In areas of high malaria transmission settings, a high prevalence of helminths co-infections can be found[219]. Infections with different helminths species alter the immune system[220] and subsequently influence vaccinations[141]. In the current study we aimed to assess the effect of chronic helminths infection on GMZ2 vaccine induced immune response. We discovered a trend towards increased vaccine specific antibodies in the helminths infected study groups compared to the non-infected. The effect is weaker for *S. haematobium* and stronger and significant for intestinal helminths especially *T. trichiura.*

Interestingly, these results contradict previous findings. Even though heterogenous effects regarding the impact of different helminths infections on vaccine induced immunity are described in the literature, overall implications lead rather to a reduced immune response to vaccines. Schistosomiasis can have a negative impact on the immune response to tetanus-toxoid[221,222], Bacillus Calmette-Guerin (BCG)[153], and hepatitis B surface antigen[222]. Or it has no or low impact on BCG, hepatitis B, or tetanus vaccinations[223,224]. An association of increased antibody response to an infection with *Schistosoma*, as it is witnessed in our study, is not described.

Regarding intestinal helminths the effects are seldom evaluated on species level. For *A. lumbricoides* a negative impact on cholera vaccines induced antibodies is shown[148,168,225]. The impact of hookworms assessed in combination with other helminths results also in impaired vaccine responses[153] or non-interference[226–228]. *T. trichiura* leads to a suppressed immune response in previous studies. A clinical trial of particular interest in this context is a former GMZ2 study conducted with a study design similar to ours[168]. Esen et al. described an impaired vaccine induced antibody response in helminths infected children. This is of interest, since in their study the helminths species with the highest impact on the induced immunogenicity was *T. trichiura.* It is the same species, which also played a predominant role in our study – solely with an opposite effect.

There are two major differences between the study of Esen et al. and our study, which may have contributed to the difference in outcome. First, the study population differed: In the current study we included semi-immune adults, whereas in the phase I trial of Esen et al. the study population was composed of children aged from one to five. Their immune

system had much less exposure to malaria parasites and other pathogens and consequently reacts different upon vaccination and immunomodulation.

Second, the selection of adjuvants: While Esen et al. evaluated GMZ2 adjuvanted with Alhydrogel, in our study 29 of 41 analyzed participants received a GMZ2-CAF01 formulation. Evidence rises from pre-clinical studies, that the helminth induced T_H2 bias and downregulation of the immune system may be overcome by the right choice of adjuvants. This is *inter alia* indicated by a study where *Schistosoma*-infected mice were vaccinated with HIV-1 immunogen either co-administered with oligodeoxynucleotides containing unmethylated cytosine–phosphate–guanosine immunostimulatory or with incomplete Freund's adjuvant. The former was able to induce potent T_H1 anti-HIV-1 immune responses whereas the latter evoked weak T_H2 marked immunogenicity[229].

One may postulate that immunization with CAF01 (T_H1 profile) adjuvanted vaccines induces a higher immune response in helminths infected humans in contrast to non-helminths infected humans: Participants could had had experienced a downregulation of immune responses due to helminths infection and consequently could had had a lower response upon natural infection ahead of the study. Therefore, they could had a lower baseline IgG. In the course of immunization with CAF01 the immune system might have been re-upregulated - especially with regards to the study vaccination antigens.

Thus, starting at a lower baseline IgG, a raise to the same IgG titers at day 84 could have led to a stronger effect in x-fold change of the vaccine specific IgG in the helminths infected group. Indeed, the mean baseline IgG of infected participants was slightly lower (figure 38, annex). However, the absolute concentration of IgG at day 84 was higher in contrast to non-infected participants (figure 39, annex).

Therefore, the higher x-fold change of vaccine specific IgG can at best be partially explained by lower baseline IgG and the downregulated immune system due to helminths infection ahead of the study.

Apart from the two major differences between the studies, there are several minor matters: E.g. Esen et al. analyzed the log-transformed AUC, whereas we assessed the x-fold change of IgG titer. However, in a second analysis the baseline corrected log transformed AUC was also calculated in this study for the sake of comparability (data not shown). The overall trend was similar though less pronounced.

Further, one question is, how long the participants have to be infected until the helminth infection has an impact on the vaccine induced immunogenicity. In our study we applied the same method as Esen et al. and assessed the infection status at two timepoints: Day 0 and day 84. For the analysis we allocated every participant in the infected group, when he was infected at day 0 and/or at day 84. Therefore, a participant, who was negative at the screening and got infected at the end of the study period still counted as infected. It could be argued, that in that case the duration of the helminth infection was not long enough to influence the immune response to the study vaccine.
Thus, the influence of helminth infections on the study vaccine was re-analyzed, while considering solely participants infected on day 0. In consequence, the number of observations was lower. Two participants were diagnosed with hookworms and three developed schistosomiasis during the study period. They were not counted as infected in the second analysis. This analysis (data not shown) showed a similar trend to higher IgG response in the infected group, even though the effect was smaller.

In conclusion, several explications can be considered for the discrepancy of our results compared to former studies. The argument of different study populations may not be suitable, since in former trials study populations consisting of semi-immune adults were present[153,221,222,227]. The same applies for variance in evaluated helminth species, since the same species were analyzed e.g. *T. trichiura* in the case of Esen et al., or *S. haematobium* in the case of Malhotra et al. A question of the variation in vaccine antigen can be diminished with regards to Esen et al. A possible partial explanation might still be the choice of adjuvant. However, random effects or undetected confounders could have also caused the difference in outcome since the study was very limited due to low observation numbers.

Further research in larger trials are needed to deepen the knowledge on the effect of Helminths infection on vaccine induced immunity. This accounts particularly for *A. lumbricoides* and *L. loa*, since the study was not powered enough to analyze their influence. This is not only of interest for further efforts on the way to a malaria vaccine, but also applies to already licensed and implemented vaccines in helminth endemic areas.

4.4 Relationship between tolerability and immunogenicity

There are several ways the immune reaction upon vaccination can manifest. Measuring the vaccine-specific antibody response is a direct approach to evaluate the immune response. However, adverse events may be induced by different aspects of the same immune reaction (e.g. local and systemic inflammatory reactions) and therefore can be part of the same immune response. I therefore hypothesized that a stronger immune reaction upon vaccination could be associated with higher antibody titer and more severe or higher frequencies of adverse events.

While looking at the tolerability and immunological profile separately, no dose-dependent variations could be demonstrated between the study groups. The association between the immune response and AEs that are at least possibly related to the investigational medicinal product did not show a robust relationship.

There are several possible explanations: Firstly, the most important limitation of this analysis is the small sample size of the clinical study and the general low frequency of AEs within the trial. Thus, the study might not be suited for detecting subtle effects. Secondly, a high local or systematic inflammatory response may lead to increased local, or systemic AEs. However, the quality of elicited antibodies may be influenced rather than the amount by the intensity of the immune reaction. Depending on the signaling of the PRRs of APCs, different cytokines are secreted, and consequently distinct T cell subsets are promoted. These in turn may or may not, depending on the subset, support the maturation of B cells and the subsequent development of antibodies[48]. Thirdly, the inflammatory immune response that generates AEs may not influence the vaccine-specific immune response.

Of note, there was a weak negative correlation between the dynamics of HGB and the IgG at day 84 ($r=-26$, $p = 0.0038$) and the x-fold change of IgG ($r=-0.26$, $p = 0.0046$). This could indicate that some participants, who eventually experienced sub-clinic malaria infections, were naturally boosted during the study period. Thus, they might have lost erythrocytes due to the erythrocytic schizogony and gained vaccine specific IgG.

4.5 Limitations of the thesis

Limited samples size

This trial was designed as a phase I clinical study. This phase represents an early stage of clinical development and focuses mainly on basic tolerability assessment. Therefore, a small number of participants were investigated. Fifty study subjects are common sample for a phase I clinical trial. The number of study subjects is suitable to assess tolerability, while not putting too many participants at unnecessary risk. Three participants did not finish their vaccination schedule, two study subjects of the rabies group and one of the GMZCAF01 group, which was correctly accounted for in the planning of the study. Therefore, the desired minimum of 11 participants in the GMZ2-Alhydrogel and 11 in the GMZ2CAF01 group as explained in the sample size justification was achieved.

Sample size with regards to safety

As to be expected in a phase I trial, the sample size was not suited to establish an extended tolerability profile for GMZ2 adjuvanted with CAF01. The number of study subjects was not enough to detect rare and Grade 3 AE. Larger follow up trials like the previous multicenter trial with 1849 participants included and over 40 reported Grade 3 AEs are necessary to establish a safe GMZ2-CA01 regime.

Sample size with regards to immunogenicity

Regarding the immunogenicity, the study was powered to detect a rather large difference between GMZ2 and the comparator vaccine. In contrast, it was not sufficient to detect a significant variation within the different GMZ2 vaccine formulations concerning different adjuvants and dosage. As the primary objective of this phase I trial was not to conduct a head to head comparison between the different vaccine formulations, further studies with larger sample size might be indicated to address this question.

Sample size with regards to helminth infection

The prevalence of helminth infection in our cohort was small, despite of the high burden of helminth infection in Lambaréné. Therefore, any conclusion drawn from this analysis has to be limited. Even though lower prevalence of helminth infection is to be expected in an adult population[230], the methods of parasitological diagnostics in this study

comprised microscopy and culture. Therefore, low-level infections might have been missed. Further, species-specific analysis was only possible for *T. trichiura*.

Assessment of immunogenicity only based on quantitative antibody assay

In the search for a potential malaria vaccine, the question of a suitable immunological substitute is left unanswered[204]. Among the tests used to detect *Plasmodium spp.* specific antibody ELISA is most common. This test is applied in most of malaria vaccine trials such as RTS,S[213], GMZ2 precursor studies[121–124], and various others. Nevertheless, the determination of vaccine induced immunogenicity by ELISA poses certain limitations. ELISA is a purely quantitative method, which does not provide insight into the functionality of elicited IgG[231]. This is of particular importance since in vivo antibodies are competing for epitopes on the merozoite resulting in various interactions and even in the blockage of effective antibodies[231]. Thus, functional antibody tests can yield a different picture regarding vaccine induced immunogenicity. In the context of malaria vaccine development, ADCI assays have been used to assess antibody function. This assay was used in previous GMZ2 studies and showed that GMZ2 vaccine induced antibodies were functional and had broad inhibitory effect[118]. Although ADCI was not used in this study, the information yield in previous trials on the functionality of the GMZ2-Alhydrogel antibodies can be extended to our finding. Nevertheless, a focus on the humoral part of the immune system leaves the question for the cellular immune system unanswered.

Another method to assess the vaccine efficacy rather directly is a CHMI. Using this technique, one does not rely on natural infection and large efficacy studies to obtain preliminary results on the vaccine induced protection. The clinical trial, which provided the data for this thesis, included such a CHMI. The results of this CHMI are discussed elsewhere[172].

Study population

Regarding the study population, our cohort consisted of young semi-immune male adults. Although the inclusion of adults is recommended for first in human trials, the data generated can present some bias as the vaccine is primarily intended for use in children below 5 years of age. Indeed, our participants had a long history of malaria transmission, developed semi-immunity and their immune system reacts differently upon vaccination. Infants have a less experienced immune system and are more likely to be malaria naïve. Nevertheless, phase I studies are focused on the establishment of a safe and tolerable vaccination regime before moving to a more vulnerable target population. Further, the experience gained so far in the clinical development of GMZ2 has shown that the trend observed in the phase I trials can be translated to a phase I study in children[121–124].

Potential malaria infection at the timepoint of vaccination

Another interesting point is, that an active malaria infection might interfere with immunization. Evidence indicates an impairment of dendritic cell function upon uptake of hemozoin pigments (parasite waste product). Further dendritic cell suffer IL-10-mediated apoptosis upon infection[35]. Consequently, antigen presentation during immunization may be compromised and therefore the induction of the adaptive immune system upon vaccination may be equally impaired. In this current study, the status of occult malaria infection was assessed via TBS during screening for all participants. The timeframe between the screening and the first vaccination was at most twenty-one days. A malaria infection may have occurred within this timeframe. Moreover, asymptomatic, submicroscopic parasitemia was not further assessed during the study period. It may have occurred at a later timepoint and interfered with the second or third immunization. Further research is needed to assess the impact of active malaria infections on vaccinations and whether a preventive anti-malarial treatment is beneficial before vaccination. It will also be important to determine the time between the treatment and immunization, because the impact of the infection will extend for a time even further after treatment.

4.6 Conclusions

In the present study we showed that the vaccine candidate GMZ2 adjuvanted with CAF01 is well tolerated and safe. The concern that the combination of GMZ2 with a more potent adjuvant like CAF01 might lead to an increased rate or severity of side effects was not confirmed.

Furthermore, GMZ2-CAF01 formulations elicited a robust immune response, which however was not superior to GMZ2-Alhydrogel. Further research is needed to deepen our knowledge on cellular responses and antibody functions, specificity, avidity, and maturation.

Helminth infection was associated with a better vaccine-specific IgG response. This observation is new and contradicts previous findings. Among others, it may be the result of a lifelong exposure to parasites in our study population. Translation of these findings have to be done with caution and it is recommended assessing this question in larger clinical trials.

In reference to the induced vaccine efficacy and immunogenicity it is unlikely, that GMZ2 formulated with CAF01 can contribute to the fight against malaria. Still, it is proven that GMZ2 can elicit functional antibodies[118] and that the vaccine efficacy increases with higher immunogenicity[124]. Thus, a different GMZ2 formulation may still be worth testing to improve vaccine efficacy. Among the potential adjuvant candidates immunopotentiating reconstituted influenza virosomes offer a promising proprietary vaccine platform[215]. An improved GMZ2 formulation may further be combined with other malaria vaccines (e.g. sexual, sporogonic or mosquito stage vaccines interrupting malaria transmission) and complement current malaria control efforts in a potential malaria eradication program.

5 Summary

5.1 English summary

Despite a vast reduction of incidence and mortality rates during the beginning of the twenty-first century, malaria continues to be a threat to mankind. Especially in the recent years achievements have plateaued. In 2017 approximately 219 million cases occurred, of which 435,000 ended fatal. The majority of deaths occur in children and pregnant women. Previous successes are fragile and current key tools against malaria (ITN, IRS, and ACT) are under constant threat from emerging resistances. Especially the increased report of resistance of *Plasmodiumm spp*. strains to anti-malarial drugs in South Asia add to the complexity of the situation and indicate the urgent need to improve the strategy to control the disease. This is endorsed by the WHO and reflected in the "*Global Technical Strategy for Malaria 2016–2030"* that recognized the urgent need and pivotal importance of developing a vaccine against malaria for successful control and possible eradication of the disease.

Until now RTS,S, a pre-erythrocytic malaria vaccine, has been the only vaccine to complete phase III development. It is currently undergoing an implementation phase IV study in three African countries after receiving scientific approval from the EMA and the WHO. Beside RTS,S, several others malaria vaccine candidates are currently assessed in clinical trials. GMZ2 is one of these vaccines and is a recombinant fusion protein consisting of conserved domains of GLURP and MSP3, two asexual blood-stage antigens of *P. falciparum*. It is designed mimicking naturally acquired anti-malarial blood-stage immunity. Clinical development of GMZ2 adjuvanted with Alhydrogel comprises several phase I trials conducted with children and adults in Africa as well as a recent phase II multi-center and multi-country trial. This phase II trial involving 1849 participants 12 to 60 months of age confirmed the good tolerability of the vaccine candidate. Even though the protection conferred by the vaccine was modest, it has demonstrated that the vaccine efficacy may be increased by improving the vaccine immunogenicity. A straight-forward approach is to modify the adjuvant in order to enhance the immunogenicity with the final aim to increase the overall vaccine efficacy. CAF01 is a novel liposomal adjuvant system inducing a robust and long lived humoral and cellular immune response characterized by a T_H1 profile. Pre-clinical head-to-head

comparison of CAF01 with Alhydrogel proved its superiority in immune enhancing properties.

The current study was designed to assess tolerability and immunogenicity of GMZ2-CAF01, as a new formulation of the GMZ2 vaccine candidate. In addition, the impact of helminth infection on vaccine induced immune response was determined. The study was designed as a randomized, double blind, single-center phase I clinical trial conducted in Lambaréné, Gabon. Fifty healthy young males with an history of at least 10 years of malaria transmission were recruited and allocated to 4 different study arms: A (Rabies, as comparator vaccine; n = 8), B (100µg GMZ2-Alhydrogel; n = 12), C (30µg GMZ2-CAF01; n = 8), and D (100µg GMZ2-CAF01; n = 22). The participants were vaccinated on D0, D28, and D56 i.m. in alternating deltoid muscles. Safety and tolerability were assessed during the follow up with non-leading questions, symptom focused clinical examination and recurrent laboratory analysis. Immunogenicity was examined through the altitude of vaccine specific immunoglobulin titers measured with ELISA. Helminth infection status was evaluated by analyzing stool and blood samples at screening and on day 84.

We confirmed that the vaccine candidate GMZ2 adjuvanted with CAF01 is well tolerated and safe. No serious or Grade 3 AE occurred. The predominant number of AEs was mild and pain at infection site. Safety signals were equivalent among study groups and GMZ2 formulations. Regarding the immunogenicity, GMZ2-CAF01 formulations elicited a robust immune response, which however was not superior to GMZ2-Alhydrogel.

Interestingly, helminth infection positively affected the altitude of vaccine specific IgG. This contradicts previous findings. So far, depending on the helminth species, rather negative implications were demonstrated if helminths infections were present during vaccination. Particularly, a GMZ2 precursor trial, conducted by Esen et al. reported reduced immune response in the presence of *T. trichiura*. Reasons for the differences in immunogenicity outcome may be the distinct study populations, since Esen et al. assessed the immune response in children, whereas this study worked with semi-immune adult subjects. Moreover, any conclusions drawn from these results is limited by the low observation numbers. To evaluate these effects further, larger clinical trials are recommended.

In reference to the induced vaccine efficacy and immunogenicity it is unlikely, that GMZ2 formulated with Alhydrogel or CAF01 can contribute to the fight against malaria. Still, it is proven that GMZ2 can elicit functional antibodies and that the vaccine efficacy increases with higher immunogenicity. Thus, a different GMZ2 formulation may still induce a sufficient vaccine efficacy. Among the potential adjuvant candidates immunopotentiating reconstituted influenza virosomes offer a promising proprietary vaccine platform. An improved GMZ2 formulation may further be combined with other malaria vaccines (e.g. sexual, sporogonic or mosquito stage vaccines interrupting malaria transmission) and complement current malaria control efforts in a potential malaria eradication program.

5.2 German summary

Trotz des Rückgangs der Inzidenz und Mortalität zu Beginn des einundzwanzigsten Jahrhunderts stellt Malaria immer noch eine der größten Bedrohungen der Menschheit dar. Gerade in den letzten fünf Jahren stagnierten die Erfolge im Kampf gegen die Malaria. 2017 wurden immer noch circa 219 Millionen Krankheitsfälle verzeichnet von denen 435.000 tödlich endeten. Besonders vulnerable Gruppen sind hierbei Kinder und Schwangere. Die bisherigen wichtigsten Maßnahmen gegen Malaria (Insektizid imprägnierte Moskitobettnetze, Versprühen von Insektiziden in Innenräumen und Artemisinin Kombinationstherapien) sind durch aufkommende Insektizid- und Medikamentenresistenzen bedroht. Besonders die vermehrten Berichte über Artemisininresistenzen in Süd-Ost-Asien unterstreichen die Notwendigkeit neue Möglichkeiten in der Malariabekämpfung zu erforschen. Dies spiegelt sich auch in der globalen technischen Strategie gegen Malaria wider, welche von der Weltgesundheitsorganisation (WHO) veröffentlich wurde. Dort wird auf die Wichtigkeit eines Malariaimpfstoffes hingewiesen, um die Reduktion und die potentielle Eradikation von Malaria zu ermöglichen.

Bislang hat nur ein Impfstoffkandidat (Mosquirix) die dritte Phase der klinischen Erprobung abgeschlossen. Der Impfstoff wird zurzeit in einer Phase IV Implementierungsstudie in drei Afrikanischen Ländern getestet, nachdem er Befürwortung durch EMA und WHO erlangt hatte. Neben diesem führenden Malaria Impfstoffkandidaten befinden sich weitere in der klinischen Erprobung. GMZ2 ist einer von diesen und besteht aus einem rekombinanten Fusionsprotein der konservativen Regionen des *Glutamat Rich Protein* (GLURP) und des *Merozoite Surface Protein 3* (MSP3). Diese sind zwei Antigene von *P. falciparum* welche primär während der erythrozytären Schizogony exprimiert werden. Der Impfstoff zielt darauf ab, die Vermehrung des Erregers im Blut zu kontrollieren und somit klinische Symptome zu verhindern oder zu mildern. GMZ2 wurde zusammen mit Alhydrogel als Adjuvant bereits in drei Phase I Studien unter anderem in semi-immunen Erwachsenen und Kindern in Sub-Sahara Afrika getestet. Zusätzlich wurde 2010 eine multizentrale Phase II Studie mit 1849 zwölf bis sechzig Monate alten Kindern durchgeführt. Auch wenn die Studie das gute Sicherheitsprofil des Impfstoffes bestätigen konnte, so war doch die

Impfwirksamkeit mit 13,6% nicht zufriedenstellend. Aber es wurde ein Zusammenhang zwischen Immunogenität und der Wirksamkeit hergestellt. Dadurch ergibt sich die Vermutung, dass eine Steigerung der Immunogenität auch eine Verbesserung der Wirksamkeit bedeutet. Eine Möglichkeit diese Steigerung durchzuführen ist durch die Auswahl eines stärkeren Adjuvanten. CAF01 ist ein potentes liposomales Adjuvant System, welches sowohl humoral wie auch zellulär eine langlebige Immunantwort auslöst. Besonders die T_H1 betonte Immunreaktion ist für einen Malariaimpfstoff wertvoll. Darüber hinaus zeigte CAF01 in einem direkten präklinischen Vergleich eine dem Aluminium überlegene Immunogenität.

Diese Dissertation basiert auf einer randomisierten Phase I Doppelblindstudie, welche durchgeführt worden ist, um die Sicherheit und Immunogenität von GMZ2 in Kombination mit CAF01 zu erfassen. Zusätzlich wurden die Auswirkungen von parasitären Konfektionen auf die Impfantwort untersucht. Für die Studie wurden 50 semi-immune männliche Erwachsene aus Lambaréné (Gabun) eingeschlossen und in vier Gruppen eingeteilt: A (Tollwutimpfstoff als Kontrollgruppe; n = 8), B (100µg GMZ2-Alhydrogel; n = 12), C (30µg GMZ2-CAF01; n = 8), und D (100µg GMZ2-CAF01; n = 22). Die Teilnehmer wurden am Tag 0, 28 und 56 in den *Musculus deltoideus* geimpft. Das Sicherheitsprofil wurde durch nicht suggestive Fragen, symptomorientierte klinische Untersuchungen und wiederholte Laboruntersuchungen erfasst. Zur Immunogenität wurden die Kozentrationen der impfstoffspezifischen Immunglobuline mittels ELISA untersucht. Ko-infektionen wurden durch Blut und Stuhlproben während des Screenings und am Tag 84 bestimmt.

Wir bestätigen, dass GMZ2-CAF01 sicher und verträglich ist. In der Studie ist weder ein schwerwiegendes unerwünschtes Ereignis aufgetreten, noch eines vom Schweregrad III. Der vorherrschende Teil der unerwünschten Ereignisse waren von mildem Schweregrad mit Fokus auf Schmerzen an der Einstichstelle. Das Sicherheitsprofil war vergleichbar zwischen den Studiengruppen. Durch die Impfung wurde eine Steigerung der impfstoffspezifischen Antikörper erzielt. Jedoch war die Immunantwort auf GMZ2-CAF01 nicht signifikant größer als die GMZ-Alhydrogel induzierte Antwort.

Interessanterweise wirkten sich die parasitären Ko-infektionen positiv auf die Immunogenität des Impfstoffes aus. Dies steht im Widerspruch zu der bisherigen

Forschung. Im Besonderen zeigte eine direkte Vorläuferstudie verminderte impfstoffspezifischen Antikörper in der Gegenwart von *T. trichiura* nach Impfung von GMZ2-Alhydrogel. Ein möglicher Grund für die unterschiedlichen Resultate können die unterschiedlichen Studienpopulationen sein. Während wir semi-immune Erwachsene impften, so wurde im Falle von Esen et al. der Impfstoff Kindern verabreicht. Zusätzlich limitiert die geringe Anzahl von Probanden die möglichen Schlüsse. Weitere, größere Studien sind nötig, um den Effekt von Konfektionen auf Malaria Impfstoffkandidaten zu untersuchen.

Im Hinblick auf die induzierte Immunantwort ist es unwahrscheinlich, das GMZ-CAF01 in seiner jetzigen Form einen Beitrag zum Kampf gegen Malaria leisten kann. Dennoch wurde in früheren Studien gezeigt, dass eine erhöhte Immunogenität auch zu einer besseren Wirksamkeit führen kann. Daher könnte sich GMZ2 in einer anderen Formulierung, wie zum Beispiel einem wirksameren Adjuvanten, immer noch als wertvoll erweisen. Unter den potentiellen Adjuvantsystemen stellen rekonstituierte Influenzavirosomen eine vielversprechende Kombinationsmöglichkeit dar.

6. References

1. Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria. *Clin Microbiol Rev*. 2002;15(4):564-594. doi:10.1128/cmr.15.4.564-594.2002

2. Institute of Medicine (U.S.), Oaks SC, eds. *Malaria: Obstacles and Opportunities: A Report of the Committee for the Study on Malaria Prevention and Control: Status Review and Alternative Strategies, Division of International Health, Institute of Medicine*. Washington, D.C: National Academy Press; 1991.

3. WHO. Eliminating malaria. April 2016. http://apps.who.int/iris/bitstream/10665/205565/1/WHO_HTM_GMP_2016.3_eng.pdf. Accessed October 1, 2016.

4. Cibulskis RE, Alonso P, Aponte J, et al. Malaria: Global progress 2000 – 2015 and future challenges. *Infect Dis Poverty*. 2016;5(1):61, s40249-016-0151-0158. doi:10.1186/s40249-016-0151-8

5. World Health Organization. *World Malaria Report 2015.* Geneva, Switzerland; 2016.

6. Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature*. 2015;526(7572):207-211. doi:10.1038/nature15535

7. Organisation mondiale de la santé. *World Malaria Report 2018*. Geneva; 2018.

8. Parpia AS, Ndeffo-Mbah ML, Wenzel NS, Galvani AP. Effects of Response to 2014–2015 Ebola Outbreak on Deaths from Malaria, HIV/AIDS, and Tuberculosis, West Africa. *Emerg Infect Dis*. 2016;22(3):433-441. doi:10.3201/eid2203.150977

9. World Health Organization, World Health Organization, eds. *Global Plan for Insecticide Resistance Management in Malaria Vectors*. Geneva, Switzerland: World Health Organization; 2012.

10. Cui L, Mharakurwa S, Ndiaye D, Rathod PK, Rosenthal PJ. Antimalarial Drug Resistance: Literature Review and Activities and Findings of the ICEMR Network. *Am J Trop Med Hyg*. 2015;93(3 Suppl):57-68. doi:10.4269/ajtmh.15-0007

11. Mosquito Malaria Vectors – Malaria Atlas Project. http://www.map.ox.ac.uk/explore/mosquito-malaria-vectors/. Accessed February 10, 2016.

12. Schantz-Dunn J, Nour NM. Malaria and Pregnancy: A Global Health Perspective. *Rev Obstet Gynecol*. 2009;2(3):186-192.

13. Caminade C, Kovats S, Rocklov J, et al. Impact of climate change on global malaria distribution. *Proc Natl Acad Sci U S A*. 2014;111(9):3286-3291. doi:10.1073/pnas.1302089111

14. Autino B, Noris A, Russo R, Castelli F. EPIDEMIOLOGY OF MALARIA IN ENDEMIC AREAS. *Mediterr J Hematol Infect Dis*. 2012;4(1):2012060. doi:10.4084/mjhid.2012.060

15. WHO | Malaria Fact sheet N°94. WHO. http://www.who.int/mediacentre/factsheets/fs094/en/. Accessed August 27, 2015.

16. Smith JD, Rowe JA, Higgins MK, Lavstsen T. Malaria's deadly grip: cytoadhesion of *Plasmodium falciparum* -infected erythrocytes: *Plasmodium falciparum* cytoadhesion and *var* genes. *Cell Microbiol*. 2013;15(12):1976-1983. doi:10.1111/cmi.12183

17. Davidson R, Brent A, Seale A, eds. *Oxford Handbook of Tropical Medicine*. Fourth edition. Oxford, United Kingdom ; New York, NY, United States of America: Oxford University Press; 2014.

18. Collins WE, Jeffery GM. Plasmodium ovale: Parasite and Disease. *Clin Microbiol Rev*. 2005;18(3):570-581. doi:10.1128/CMR.18.3.570-581.2005

19. Zaw MT, Lin Z. Two sympatric types of Plasmodium ovale and discrimination by molecular methods. *J Microbiol Immunol Infect*. 2017;50(5):559-564. doi:10.1016/j.jmii.2016.08.004

20. Baird JK. Evidence and Implications of Mortality Associated with Acute Plasmodium vivax Malaria. *Clin Microbiol Rev*. 2013;26(1):36-57. doi:10.1128/CMR.00074-12

21. Anstey NM, Douglas NM, Poespoprodjo JR, Price RN. Plasmodium vivax. In: *Advances in Parasitology*. Vol 80. Elsevier; 2012:151-201. doi:10.1016/B978-0-12-397900-1.00003-7

22. Collins WE, Jeffery GM. Plasmodium malariae: Parasite and Disease. *Clin Microbiol Rev*. 2007;20(4):579-592. doi:10.1128/CMR.00027-07

23. Wesolowski R, Wozniak A, Mila-Kierzenkowska C, Szewczyk-Golec K. Plasmodium knowlesi as a Threat to Global Public Health. *Korean J Parasitol*. 2015;53(5):575-581. doi:10.3347/kjp.2015.53.5.575

24. Prevention C-C for DC and. CDC - Malaria - About Malaria - Biology. https://www.cdc.gov/malaria/about/biology/index.html. Published January 28, 2019. Accessed June 6, 2019.

25. Mackintosh CL, Beeson JG, Marsh K. Clinical features and pathogenesis of severe malaria. *Trends Parasitol*. 2004;20(12):597-603. doi:10.1016/j.pt.2004.09.006

26. Patwardhan B, Chaguturu R. *Innovative Approaches in Drug Discovery: Ethnopharmacology, Systems Biology and Holistic Targeting*. Academic Press; 2016.

27. Curtidor H, Patarroyo ME, Patarroyo MA. Recent advances in the development of a chemically synthesised anti-malarial vaccine. *Expert Opin Biol Ther*. 2015;15(11):1567-1581. doi:10.1517/14712598.2015.1075505

28. Malaguarnera L, Musumeci S. The immune response to Plasmodium falciparum malaria. *Lancet Infect Dis*. 2002;2(8):472-478. doi:10.1016/S1473-3099(02)00344-4

29. Langhorne J, Ndungu FM, Sponaas A-M, Marsh K. Immunity to malaria: more questions than answers. *Nat Immunol*. 2008;9(7):725-732. doi:10.1038/ni.f.205

30. Doolan DL, Dobano C, Baird JK. Acquired Immunity to Malaria. *Clin Microbiol Rev*. 2009;22(1):13-36. doi:10.1128/CMR.00025-08

31. Riley EM, Stewart VA. Immune mechanisms in malaria: new insights in vaccine development. *Nat Med*. 2013;19(2):168-178. doi:10.1038/nm.3083

32. Templeton TJ. The varieties of gene amplification, diversification and hypervariability in the human malaria parasite, Plasmodium falciparum. *Mol Biochem Parasitol*. 2009;166(2):109-116. doi:10.1016/j.molbiopara.2009.04.003

33. Mackinnon MJ, Marsh K. The Selection Landscape of Malaria Parasites. *Science*. 2010;328(5980):866-871. doi:10.1126/science.1185410

34. Ouattara A, Laurens MB. Vaccines Against Malaria. *Clin Infect Dis*. 2015;60(6):930-936. doi:10.1093/cid/ciu954

35. Stanisic DI, Barry AE, Good MF. Escaping the immune system: How the malaria parasite makes vaccine development a challenge. *Trends Parasitol*. 2013;29(12):612-622. doi:10.1016/j.pt.2013.10.001

36. Guilbride DL, Gawlinski P, Guilbride PDL. Why Functional Pre-Erythrocytic and Bloodstage Malaria Vaccines Fail: A Meta-Analysis of Fully Protective Immunizations and Novel Immunological Model. *PLoS ONE*. 2010;5(5). doi:10.1371/journal.pone.0010685

37. Finney OC, Riley EM, Walther M. Regulatory T cells in malaria – friend or foe? *Trends Immunol*. 2010;31(2):63-70. doi:10.1016/j.it.2009.12.002

38. Niewold P, Cohen A, Vreden C van, Getts DR, Grau GE, King NJC. Experimental severe malaria is resolved by targeting newly-identified monocyte subsets using immune-modifying particles combined with artesunate. *Commun Biol*. 2018;1(1):1-13. doi:10.1038/s42003-018-0216-2

39. Pérignon JL, Druilhe P, Pérignon JL, Druilhe P. Immune mechanisms underlying the premunition against Plasmodium falciparum malaria. *Mem Inst Oswaldo Cruz*. 1994;89:51-53. doi:10.1590/S0074-02761994000600013

40. Cohen S, McGREGOR IA, Carrington S. Gamma-globulin and acquired immunity to human malaria. *Nature*. 1961;192:733-737.

41. Sabchareon A, Burnouf T, Ouattara D, et al. Parasitologic and Clinical Human Response to Immunoglobulin Administration in Falciparum Malaria. *Am J Trop Med Hyg*. 1991;45(3):297-308. doi:10.4269/ajtmh.1991.45.297

42. Bouharoun-Tayoun, H., Phanorsi Attanath, Sabchareon A., Chongsuphajaisiddhi T. Antibodies that protect humans against Plasmodium falciparum blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes. *J Exp Med*. 1990;172(6):1633-1641.

43. Dolo A, Modiano D, Maiga B, et al. Difference in susceptibility to malaria between two sympatric ethnic groups in Mali. *Am J Trop Med Hyg*. 2005;72(3):243–248.

44. Marsh K, Kinyanjui S. Immune effector mechanisms in malaria. *Parasite Immunol*. 2006;28(1-2):51-60. doi:10.1111/j.1365-3024.2006.00808.x

45. Garraud O, Mahanty S, Perraut R. Malaria-specific antibody subclasses in immune individuals: a key source of information for vaccine design. *Trends Immunol*. 2003;24(1):30-35. doi:10.1016/S1471-4906(02)00012-1

46. Schwenk RJ, Richie TL. Protective immunity to pre-erythrocytic stage malaria. *Trends Parasitol*. 2011;27(7):306-314. doi:10.1016/j.pt.2011.02.002

47. Wipasa J, Elliott S, Xu H, Good MF. Immunity to asexual blood stage malaria and vaccine approaches. *Immunol Cell Biol*. 2002;80(5):401-414. doi:10.1046/j.1440-1711.2002.01107.x

48. Murphy K, Weaver C. *Janeway's Immunobiology*. 9th edition. New York, NY: Garland Science/Taylor & Francis Group, LLC; 2016.

49. Soon MSF, Haque A. Recent Insights into CD4+ Th Cell Differentiation in Malaria. *J Immunol*. 2018;200(6):1965-1975. doi:10.4049/jimmunol.1701316

50. Löscher T, Burchard G-D, Lang W, Bendick C, eds. *Tropenmedizin in Klinik und Praxis: mit Reise- und Migrationsmedizin ; 287 Tabellen ; [mit Online-Zusatzmaterial]*. 4., komplett überarb. und erw. Aufl. Stuttgart: Thieme; 2010.

51. Wiley J. Severe Malaria. *Trop Med Int Health*. 2014;19:7-131. doi:10.1111/tmi.12313_2

52. Prevention C-C for DC and. CDC - Malaria - About Malaria - Disease. http://www.cdc.gov/malaria/about/disease.html. Accessed March 9, 2016.

53. World Health Organization, Global Malaria Programme. *Guidelines for the Treatment of Malaria.*; 2015.

54. World Health Organization. *Management of Severe Malaria: A Practical Handbook.* Geneva: World Health Organization; 2012.

55. Laishram DD, Sutton PL, Nanda N, et al. The complexities of malaria disease manifestations with a focus on asymptomatic malaria. *Malar J*. 2012;11:29. doi:10.1186/1475-2875-11-29

56. Lindblade KA, Steinhardt L, Samuels A, Kachur SP, Slutsker L. The silent threat: asymptomatic parasitemia and malaria transmission. *Expert Rev Anti Infect Ther*. 2013;11(6):623-639. doi:10.1586/eri.13.45

57. Amir A, Cheong F-W, De Silva JR, Lau Y-L. Diagnostic tools in childhood malaria. *Parasit Vectors*. 2018;11(1):53. doi:10.1186/s13071-018-2617-y

58. Gendrot M, Fawaz R, Dormoi J, Madamet M, Pradines B. Genetic diversity and deletion of Plasmodium falciparum histidine-rich protein 2 and 3: a threat to diagnosis of P. falciparum malaria. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2019;25(5):580-585. doi:10.1016/j.cmi.2018.09.009

59. Organisation mondiale de la santé, Bureau régional du Pacifique occidental. *Méthodologie de la recherche dans le domaine de la santé guide de formation aux méthodes de la recherche scientifique*. Manille: Organisation mondiale de la santé; 2003.

60. Flannery EL, Chatterjee AK, Winzeler EA. Antimalarial drug discovery — approaches and progress towards new medicines. *Nat Rev Microbiol*. 2013;11(12):849-862. doi:10.1038/nrmicro3138

61. Ashley EA, Recht J, White NJ. Primaquine: the risks and the benefits. *Malar J*. 2014;13. doi:10.1186/1475-2875-13-418

62. Poumerol G, Wilder-Smith A, World Health Organization. *International Travel and Health: Situation as on January 2012.* Geneva: World Health Organization; 2012.

63. Schlitzer M. Malaria Chemotherapeutics Part I: History of Antimalarial Drug Development, Currently Used Therapeutics, and Drugs in Clinical Development. *ChemMedChem*. 2007;2(7):944-986. doi:10.1002/cmdc.200600240

64. Wani WA, Jameel E, Baig U, Mumtazuddin S, Hun LT. Ferroquine and its derivatives: New generation of antimalarial agents. *Eur J Med Chem*. 2015;101:534-551. doi:10.1016/j.ejmech.2015.07.009

65. Jones RA, Panda SS, Hall CD. Quinine conjugates and quinine analogues as potential antimalarial agents. *Eur J Med Chem*. 2015;97:335-355. doi:10.1016/j.ejmech.2015.02.002

66. Imwong M, Suwannasin K, Kunasol C, et al. The spread of artemisinin-resistant Plasmodium falciparum in the Greater Mekong subregion: a molecular epidemiology observational study. *Lancet Infect Dis*. 2017;17(5):491-497. doi:10.1016/S1473-3099(17)30048-8

67. Phyo AP, Nkhoma S, Stepniewska K, et al. Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. *Lancet*. 2012;379(9830):1960-1966. doi:10.1016/S0140-6736(12)60484-X

68. Ashley EA, Dhorda M, Fairhurst RM, et al. Spread of Artemisinin Resistance in *Plasmodium falciparum* Malaria. *N Engl J Med*. 2014;371(5):411-423. doi:10.1056/NEJMoa1314981

69. Carrara VI, Lwin KM, Phyo AP, et al. Malaria Burden and Artemisinin Resistance in the Mobile and Migrant Population on the Thai–Myanmar Border, 1999–2011: An Observational Study. *PLoS Med*. 2013;10(3). doi:10.1371/journal.pmed.1001398

70. World Health Organization, World Health Organization, Global Malaria Programme. *Global Technical Strategy for Malaria, 2016-2030*.; 2015.

71. Moorthy VS, Newman RD, Okwo-Bele J-M. Malaria vaccine technology roadmap. *The Lancet*. 2013;382(9906):1700–1701.

72. Schuchat A. Human Vaccines and Their Importance to Public Health. *Procedia Vaccinol*. 2011;5:120-126. doi:10.1016/j.provac.2011.10.008

73. John J. Role of injectable and oral polio vaccines in polio eradication. *Expert Rev Vaccines*. 2009;8(1):5-8. doi:10.1586/14760584.8.1.5

74. Henderson DA. Principles and lessons from the smallpox eradication programme. *Bull World Health Organ*. 1987;65(4):535-546.

75. Moss WJ, Griffin DE. Global measles elimination. *Nat Rev Microbiol*. 2006;4(12):900-908. doi:10.1038/nrmicro1550

76. Clyde DF, Most H, McCarthy VC, Vanderberg JP. Immunization of man against sporozite-induced falciparum malaria. *Am J Med Sci*. 1973;266(3):169-177.

77. WHO | Tables of malaria vaccine projects globally. WHO. http://www.who.int/immunization/research/development/Rainbow_tables/en/. Accessed August 20, 2019.

78. Stanisic DI, Good MF. Whole organism blood stage vaccines against malaria. *Vaccine*. 2015;33(52):7469-7475. doi:10.1016/j.vaccine.2015.09.057

79. Sergent, E., Sergent, E. Sur l'immunité dans le paludisme des Oiseaux. Conservation in vitro des sporozoites de Plasmodium relictum. Immunité relative obtenue par inoculation de ces sporozoite. *Comptes Rendus Hebd Séances L'Académie Sci*. 1910;(1511910):407-409.

80. Mordmüller B, Surat G, Lagler H, et al. Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. *Nature*. 2017;542(7642):445-449. doi:10.1038/nature21060

81. Greenwood B. Progress with the PfSPZ Vaccine for malaria. *Lancet Infect Dis*. February 2017. doi:10.1016/S1473-3099(17)30105-6

82. The RTS,S Clinical Trials Partnership. First Results of Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Children. *N Engl J Med*. 2011;365(20):1863-1875. doi:10.1056/NEJMoa1102287

83. European Medicines Agency EMA. First malaria vaccine receives positive scientific opinion from EMA. July 2015. http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2015/07/WC500190447.pdf. Accessed October 12, 2016.

84. Organization WH. Malaria vaccine: WHO position paper, January 2016–recommendations. *Vaccine*. 2017.

85. Olotu A, Fegan G, Wambua J, et al. Seven-Year Efficacy of RTS,S/AS01 Malaria Vaccine among Young African Children. *N Engl J Med*. 2016;374(26):2519-2529. doi:10.1056/NEJMoa1515257

86. Sauerwein RW, Bousema T. Transmission blocking malaria vaccines: Assays and candidates in clinical development. *Vaccine*. 2015;33(52):7476-7482. doi:10.1016/j.vaccine.2015.08.073

87. PATH's MALARIA VACCINE INITIATIVE (MVI). TBV Issue Brief. http://www.malariavaccine.org/sites/www.malariavaccine.org/files/content/resource/files/MVI-TBVfactsheetFINAL_20160420.pdf. Published April 2016. Accessed March 6, 2017.

88. WHO. Malaria transmission blocking vaccines: an ideal public good. http://www.who.int/tdr/publications/documents/malaria-transmission-blocking.pdf?ua=1. Published 2000. Accessed December 6, 2016.

89. Chaturvedi N, Bharti P, Tiwari A, Singh N. Strategies & recent development of transmission-blocking vaccines against Plasmodium falciparum. *Indian J Med Res*. 2016;143(6):696. doi:10.4103/0971-5916.191927

90. Nahrendorf W, Scholzen A, Sauerwein RW, Langhorne J. Cross-stage immunity for malaria vaccine development. *Vaccine*. 2015;33(52):7513-7517. doi:10.1016/j.vaccine.2015.09.098

91. Hill AVS. Vaccines against malaria. *Philos Trans R Soc B Biol Sci*. 2011;366(1579):2806-2814. doi:10.1098/rstb.2011.0091

92. Forbes EK, Biswas S, Collins KA, Gilbert SC, Hill AVS, Draper SJ. Combining Liver- and Blood-Stage Malaria Viral Vectored Vaccines: Investigating Mechanisms of CD8+ T cell Interference. *J Immunol Baltim Md 1950*. 2011;187(7):3738-3750. doi:10.4049/jimmunol.1003783

93. Ndam NT, Deloron P. Towards a vaccine against pregnancy-associated malaria. *Parasite*. 2008;15(3):515-521. doi:10.1051/parasite/2008153515

94. Salanti A, Dahlbäck M, Turner L, et al. Evidence for the Involvement of VAR2CSA in Pregnancy-associated Malaria. *J Exp Med*. 2004;200(9):1197-1203. doi:10.1084/jem.20041579

95. Mordmüller B, Sulyok M, Egger-Adam D, et al. First-in-human, randomized, double-blind clinical trial of differentially adjuvanted PAMVAC, a vaccine candidate to prevent pregnancy-associated malaria. *Clin Infect Dis Off Publ Infect Dis Soc Am*. January 2019. doi:10.1093/cid/ciy1140

96. Theisen M, Soe S, Brunstedt K, et al. A Plasmodium falciparum GLURP–MSP3 chimeric protein; expression in Lactococcus lactis, immunogenicity and induction of biologically active antibodies. *Vaccine*. 2004;22(9–10):1188-1198. doi:10.1016/j.vaccine.2003.09.017

97. Stricker K de, Vuust J, Jepsen S, Oeuvray C, Theisen M. Conservation and heterogeneity of the glutamate-rich protein (GLURP) among field isolates and laboratory lines of Plasmodium falciparum. *Mol Biochem Parasitol*. 2000;111(1):123-130. doi:10.1016/S0166-6851(00)00304-2

98. Singh S, Soe S, Mejia J-P, et al. Identification of a Conserved Region of Plasmodium falciparum MSP3 Targeted by Biologically Active Antibodies to Improve Vaccine Design. *J Infect Dis*. 2004;190(5):1010-1018. doi:10.1086/423208

99. Huber W, Felger I, Matile H, Joachim Lipps H, Steiger S, Beck H-P. Limited sequence polymorphism in the Plasmodium falciparum merozoite surface protein 31. *Mol Biochem Parasitol*. 1997;87(2):231-234. doi:10.1016/S0166-6851(97)00067-4

100. McColl DJ, Anders RF. Conservation of structural motifs and antigenic diversity in the Plasmodium falciparum merozoite surface protein-3 (MSP-3)1. *Mol Biochem Parasitol*. 1997;90(1):21-31. doi:10.1016/S0166-6851(97)00130-8

101. Oeuvray C, Bouharoun-Tayoun H, Gras-Masse H, et al. Merozoite surface protein-3: a malaria protein inducing antibodies that promote Plasmodium falciparum killing by cooperation with blood monocytes. *Blood*. 1994;84(5):1594-1602.

102. Theisen M, Adu B, Mordmüller B, Singh S. The GMZ2 malaria vaccine: from concept to efficacy in humans. *Expert Rev Vaccines*. 2017;16(9):907-917. doi:10.1080/14760584.2017.1355246

103. Ogutu BR, Apollo OJ, McKinney D, et al. Blood Stage Malaria Vaccine Eliciting High Antigen-Specific Antibody Concentrations Confers No Protection to Young Children in Western Kenya. *PLoS ONE*. 2009;4(3). doi:10.1371/journal.pone.0004708

104. Sagara I, Dicko A, Ellis RD, et al. A Randomized Controlled Phase 2 Trial of the Blood Stage AMA1-C1/Alhydrogel Malaria Vaccine in Children in Mali. *Vaccine*. 2009;27(23):3090-3098. doi:10.1016/j.vaccine.2009.03.014

105. Genton B, Betuela I, Felger I, et al. A Recombinant Blood-Stage Malaria Vaccine Reduces Plasmodium falciparum Density and Exerts Selective Pressure on Parasite Populations in a Phase 1-2b Trial in Papua New Guinea. *J Infect Dis*. 2002;185(6):820-827. doi:10.1086/339342

106. Thera MA, Doumbo OK, Coulibaly D, et al. A Field Trial to Assess a Blood-Stage Malaria Vaccine. *N Engl J Med*. 2011;365(11):1004-1013. doi:10.1056/NEJMoa1008115

107. Borre MB, Dziegiel M, Høgh B, et al. Primary structure and localization of a conserved immunogenic Plasmodium falciparum glutamate rich protein (GLURP) expressed in both the preerythrocytic and erythrocytic stages of the vertebrate life cycle. *Mol Biochem Parasitol*. 1991;49(1):119-131.

108. Carvalho LJM, Alves FA, Bianco C, et al. Immunization of Saimiri sciureus Monkeys with a Recombinant Hybrid Protein Derived from the Plasmodium falciparum Antigen Glutamate-Rich Protein and Merozoite Surface Protein 3 Can Induce Partial Protection with Freund and Montanide ISA720 Adjuvants. *Clin Diagn Lab Immunol*. 2005;12(2):242-248. doi:10.1128/CDLI.12.2.242-248.2005

109. Soe S, Theisen M, Roussilhon C, Aye K-S-, Druilhe P. Association between Protection against Clinical Malaria and Antibodies to Merozoite Surface Antigens in an Area of Hyperendemicity in Myanmar: Complementarity between Responses to Merozoite Surface Protein 3 and the 220-Kilodalton Glutamate-Rich Protein. *Infect Immun*. 2004;72(1):247-252. doi:10.1128/IAI.72.1.247-252.2004

110. Meraldi V, Nebié I, Tiono AB, et al. Natural antibody response to Plasmodium falciparum Exp-1, MSP-3 and GLURP long synthetic peptides and association with protection. *Parasite Immunol*. 2004;26(6-7):265-272. doi:10.1111/j.0141-9838.2004.00705.x

111. Roussilhon C, Oeuvray C, Müller-Graf C, et al. Long-Term Clinical Protection from Falciparum Malaria Is Strongly Associated with IgG3 Antibodies to Merozoite Surface Protein 3. *PLoS Med*. 2007;4(11). doi:10.1371/journal.pmed.0040320

112. Oeuvray C, Theisen M, Rogier C, Trape J-F, Jepsen S, Druilhe P. Cytophilic Immunoglobulin Responses to Plasmodium falciparum Glutamate-Rich Protein Are Correlated with Protection against Clinical Malaria in Dielmo, Senegal. *Infect Immun*. 2000;68(5):2617-2620.

113. Nebie I, Diarra A, Ouedraogo A, et al. Humoral Responses to Plasmodium falciparum Blood-Stage Antigens and Association with Incidence of Clinical Malaria in Children Living in an Area of Seasonal Malaria Transmission in Burkina Faso, West Africa. *Infect Immun*. 2008;76(2):759-766. doi:10.1128/IAI.01147-07

114. Bouharoun-Tayoun, Oeuvray C, Lunel F, Druilhe P. Mechanisms underlying the monocyte-mediated antibody-dependent killing of Plasmodium falciparum asexual blood stages. *J Exp Med*. 1995;182(2):409-418.

115. Lunel F, Druilhe P. Effector cells involved in nonspecific and antibody-dependent mechanisms directed against Plasmodium falciparum blood stages in vitro. *Infect Immun*. 1989;57(7):2043-2049.

116. Khusmith S, Druilhe P. Antibody-dependent ingestion of P. falciparum merozoites by human blood monocytes. *Parasite Immunol*. 1983;5(4):357-368. doi:10.1111/j.1365-3024.1983.tb00751.x

117. Bouharoun-Tayoun H, Druilhe P. Antibody-Dependent Cell-Mediated Inhibition (ADCI) of Plasmodium falciparum: One- and Two-Step ADCI Assays. In: Vaughan A, ed. *Malaria Vaccines*. Methods in Molecular Biology. Springer New York; 2015:131-144. doi:10.1007/978-1-4939-2815-6_11

118. Jepsen MPG, Jogdand PS, Singh SK, et al. The Malaria Vaccine Candidate GMZ2 Elicits Functional Antibodies in Individuals From Malaria Endemic and Non-Endemic Areas. *J Infect Dis*. 2013;208(3):479-488. doi:10.1093/infdis/jit185

119. Theisen M, Soe S, Oeuvray C, et al. The Glutamate-Rich Protein (GLURP) of Plasmodium falciparum Is a Target for Antibody-Dependent Monocyte-Mediated Inhibition of Parasite Growth In Vitro. *Infect Immun*. 1998;66(1):11-17.

120. Sirima SB, Cousens S, Druilhe P. Protection against Malaria by MSP3 Candidate Vaccine. *N Engl J Med*. 2011;365(11):1062-1064. doi:10.1056/NEJMc1100670

121. Esen M, Kremsner PG, Schleucher R, et al. Safety and immunogenicity of GMZ2 — a MSP3–GLURP fusion protein malaria vaccine candidate. *Vaccine*. 2009;27(49):6862-6868. doi:10.1016/j.vaccine.2009.09.011

122. Mordmüller B, Szywon K, Greutelaers B, et al. Safety and immunogenicity of the malaria vaccine candidate GMZ2 in malaria-exposed, adult individuals from Lambaréné, Gabon. *Vaccine*. 2010;28(41):6698-6703. doi:10.1016/j.vaccine.2010.07.085

123. Bélard S, Issifou S, Hounkpatin AB, et al. A Randomized Controlled Phase Ib Trial of the Malaria Vaccine Candidate GMZ2 in African Children. *PLoS ONE*. 2011;6(7). doi:10.1371/journal.pone.0022525

124. Sirima SB, Mordmüller B, Milligan P, et al. A phase 2b randomized, controlled trial of the efficacy of the GMZ2 malaria vaccine in African children. *Vaccine*. 2016;34(38):4536-4542. doi:10.1016/j.vaccine.2016.07.041

125. Organization WH, others. WHO preferred product characteristics (PPC) for malaria vaccines. 2014. http://apps.who.int/iris/handle/10665/149822. Accessed October 4, 2016.

126. Wu Y, Narum DL, Fleury S, Jennings G, Yadava A. Particle-Based Platforms for Malaria Vaccines. *Vaccine*. 2015;33(52):7518-7524. doi:10.1016/j.vaccine.2015.09.097

127. Reed SG, Orr MT, Fox CB. Key roles of adjuvants in modern vaccines. *Nat Med*. 2013;19(12):1597-1608. doi:10.1038/nm.3409

128. Clements CJ, Griffiths E. The global impact of vaccines containing aluminium adjuvants. *Vaccine*. 2002;20, Supplement 3:S24-S33. doi:10.1016/S0264-410X(02)00168-8

129. Coler RN, Carter D, Friede M, Reed SG. Adjuvants for malaria vaccines. *Parasite Immunol*. 2009;31(9):520-528. doi:10.1111/j.1365-3024.2009.01142.x

130. Perez-Mazliah D, Langhorne J. CD4 T-Cell Subsets in Malaria: TH1/TH2 Revisited. *Front Immunol*. 2015;5. doi:10.3389/fimmu.2014.00671

131. Agger EM, Rosenkrands I, Hansen J, et al. Cationic Liposomes Formulated with Synthetic Mycobacterial Cordfactor (CAF01): A Versatile Adjuvant for Vaccines with Different Immunological Requirements. *PLoS ONE*. 2008;3(9). doi:10.1371/journal.pone.0003116

132. Gordon DM, McGovern TW, Krzych U, et al. Safety, immunogenicity, and efficacy of a recombinantly produced Plasmodium falciparum circumsporozoite protein-hepatitis B surface antigen subunit vaccine. *J Infect Dis*. 1995;171(6):1576-1585.

133. Concept and results - Statens Serum Institut. http://www.ssi.dk/English/RandD/Research%20Departments/Department%20of%20Infectious%20Disease%20Immunology/Vaccine%20Adjuvant%20Research/Concept%20and%20results.aspx. Accessed August 31, 2015.

134. Christensen D, Agger EM, Andreasen LV, Kirby D, Andersen P, Perrie Y. Liposome-based cationic adjuvant formulations (CAF): Past, present, and future. *J Liposome Res*. 2009;19(1):2-11. doi:10.1080/08982100902726820

135. van Dissel JT, Joosten SA, Hoff ST, et al. A novel liposomal adjuvant system, CAF01, promotes long-lived Mycobacterium tuberculosis-specific T-cell responses in human. *Vaccine*. 2014;32(52):7098-7107. doi:10.1016/j.vaccine.2014.10.036

136. Román VRG, Jensen KJ, Jensen SS, et al. Therapeutic vaccination using cationic liposome-adjuvanted HIV type 1 peptides representing HLA-supertype-restricted subdominant T cell epitopes: safety, immunogenicity, and feasibility in Guinea-Bissau. *AIDS Res Hum Retroviruses*. 2013;29(11):1504-1512. doi:10.1089/AID.2013.0076

137. Karlsson I, Brandt L, Vinner L, et al. Adjuvanted HLA-supertype restricted subdominant peptides induce new T-cell immunity during untreated HIV-1-infection. *Clin Immunol*. 2013;146(2):120-130. doi:10.1016/j.clim.2012.12.005

138. Nacher M. Malaria vaccine trials in a wormy world. *Trends Parasitol*. 2001;17(12):563-565. doi:10.1016/S1471-4922(01)02117-1

139. Pasetti MF, Simon JK, Sztein MB, Levine MM. Immunology of Gut Mucosal Vaccines. *Immunol Rev*. 2011;239(1):125-148. doi:10.1111/j.1600-065X.2010.00970.x

140. Sack DA, Qadri F, Svennerholm A-M. Determinants of Responses to Oral Vaccines in Developing Countries. *Ann Nestlé Engl Ed*. 2008;66(2):71-79. doi:10.1159/000129624

141. Parker EP, Ramani S, Lopman BA, et al. Causes of impaired oral vaccine efficacy in developing countries. *Future Microbiol*. 2017;13(1):97-118. doi:10.2217/fmb-2017-0128

142. Edwards KM. Maternal antibodies and infant immune responses to vaccines. *Vaccine*. 2015;33(47):6469-6472. doi:10.1016/j.vaccine.2015.07.085

143. Niewiesk S. Maternal Antibodies: Clinical Significance, Mechanism of Interference with Immune Responses, and Possible Vaccination Strategies. *Front Immunol*. 2014;5. doi:10.3389/fimmu.2014.00446

144. Rytter MJH, Kolte L, Briend A, Friis H, Christensen VB. The Immune System in Children with Malnutrition—A Systematic Review. *PLoS ONE*. 2014;9(8). doi:10.1371/journal.pone.0105017

145. Prendergast AJ. Malnutrition and vaccination in developing countries. *Philos Trans R Soc B Biol Sci*. 2015;370(1671). doi:10.1098/rstb.2014.0141

146. Patriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. *Rev Infect Dis*. 1991;13(5):926-939.

147. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. *BMC Biol*. 2010;8:129. doi:10.1186/1741-7007-8-129

148. Cooper PJ, Chico ME, Losonsky G, et al. Albendazole treatment of children with ascariasis enhances the vibriocidal antibody response to the live attenuated oral cholera vaccine CVD 103-HgR. *J Infect Dis*. 2000;182(4):1199-1206. doi:10.1086/315837

149. Patel M, Shane AL, Parashar UD, Jiang B, Gentsch JR, Glass RI. Oral Rotavirus Vaccines: How Well Will They Work Where They Are Needed Most? *J Infect Dis*. 2009;200(0 1):S39-S48. doi:10.1086/605035

150. Grassly NC, Wenger J, Durrani S, et al. Protective efficacy of a monovalent oral type 1 poliovirus vaccine: a case-control study. *Lancet Lond Engl*. 2007;369(9570):1356-1362. doi:10.1016/S0140-6736(07)60531-5

151. Elias D, Akuffo H, Pawlowski A, Haile M, Schön T, Britton S. Schistosoma mansoni infection reduces the protective efficacy of BCG vaccination against virulent Mycobacterium tuberculosis. *Vaccine*. 2005;23(11):1326-1334. doi:10.1016/j.vaccine.2004.09.038

152. Urban JF, Steenhard NR, Solano-Aguilar GI, et al. Infection with parasitic nematodes confounds vaccination efficacy. *Vet Parasitol*. 2007;148(1):14-20. doi:10.1016/j.vetpar.2007.05.006

153. Elias D, Wolday D, Akuffo H, Petros B, Bronner U, Britton S. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette–Guérin (BCG) vaccination. *Clin Exp Immunol*. 2001;123(2):219-225. doi:10.1046/j.1365-2249.2001.01446.x

154. LaBeaud AD, Malhotra I, King MJ, King CL, King CH. Do Antenatal Parasite Infections Devalue Childhood Vaccination? *PLoS Negl Trop Dis*. 2009;3(5):e442. doi:10.1371/journal.pntd.0000442

155. Nookala S, Srinivasan S, Kaliraj P, Narayanan RB, Nutman TB. Impairment of Tetanus-Specific Cellular and Humoral Responses following Tetanus Vaccination in Human Lymphatic Filariasis. *Infect Immun*. 2004;72(5):2598-2604. doi:10.1128/IAI.72.5.2598-2604.2004

156. Troy SB, Rossheim AEB, Siik J, Cunningham TD, Kerry JA. Association of CMV, HBV, or HCV co-infection with vaccine response in adults with well-controlled HIV infection. *Hum Vaccines Immunother*. 2016;12(5):1295-1299. doi:10.1080/21645515.2015.1121336

157. Soil-transmitted helminth infections. World Health Organization. http://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections. Accessed June 30, 2018.

158. Mwangi TW, Bethony JM, Brooker S. Malaria and helminth interactions in humans: an epidemiological viewpoint. *Ann Trop Med Parasitol*. 2006;100(7):551-570. doi:10.1179/136485906X118468

159. Brooker S, Akhwale W, Pullan R, et al. Epidemiology of plasmodium-helminth co-infection in Africa: populations at risk, potential impact on anemia, and prospects for combining control. *Am J Trop Med Hyg*. 2007;77(6 Suppl):88–98.

160. Brooker S. Spatial epidemiology of human schistosomiasis in Africa: risk models, transmission dynamics and control. *Trans R Soc Trop Med Hyg*. 2007;101(1):1. doi:10.1016/j.trstmh.2006.08.004

161. Schistosomiasis. World Health Organization. http://www.who.int/news-room/fact-sheets/detail/schistosomiasis. Accessed June 30, 2018.

162. Metzger WG, Mordmüller B. Loa loa—Does it deserve to be neglected? *Lancet Infect Dis*. 2014;14(4):353–357.

163. BOURKE CD, MAIZELS RM, MUTAPI F. Acquired immune heterogeneity and its sources in human helminth infection. *Parasitology*. 2011;138(2):139-159. doi:10.1017/S0031182010001216

164. Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD, Allen JE. Helminth parasites – masters of regulation. *Immunol Rev*. 2004;201(1):89-116. doi:10.1111/j.0105-2896.2004.00191.x

165. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol*. 2003;3(9):733-744. doi:10.1038/nri1183

166. Zakeri A. Helminth-induced apoptosis: a silent strategy for immunosuppression. *Parasitology*. 2017;144(13):1663-1676. doi:10.1017/S0031182017000841

167. van Riet E, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections induce immunomodulation: consequences and mechanisms. *Immunobiology*. 2007;212(6):475-490. doi:10.1016/j.imbio.2007.03.009

168. Esen M, Mordmüller B, de Salazar PM, et al. Reduced antibody responses against Plasmodium falciparum vaccine candidate antigens in the presence of Trichuris trichiura. *Vaccine*. 2012;30(52):7621-7624. doi:10.1016/j.vaccine.2012.10.026

169. Keusch GT, Cash RA. A Vaccine against Rotavirus — When is Too Much Too Much? *N Engl J Med*. 1997;337(17):1228-1229. doi:10.1056/NEJM199710233371709

170. Cruz-Chan JV, Rosado-Vallado M, Dumonteil E. Malaria vaccine efficacy: overcoming the helminth hurdle. *Expert Rev Vaccines*. 2010;9(7):707-711. doi:10.1586/erv.10.63

171. Su Z, Segura M, Stevenson MM. Reduced Protective Efficacy of a Blood-Stage Malaria Vaccine by Concurrent Nematode Infection. *Infect Immun*. 2006;74(4):2138-2144. doi:10.1128/IAI.74.4.2138-2144.2006

172. Dejon-Agobe JC, Ateba-Ngoa U, Lalremruata A, et al. Controlled Human Malaria Infection of Healthy Adults With Lifelong Malaria Exposure to Assess Safety, Immunogenicity, and Efficacy of the Asexual Blood Stage Malaria Vaccine Candidate GMZ2. *Clin Infect Dis*. December 2018. doi:10.1093/cid/ciy1087

173. Stoffel D. Personal e-mail correspondence. March 2019.

174. Gabon | CIA World Factbook - The best country factbook available online. http://www.ciaworldfactbook.us/africa/gabon.html. Accessed February 3, 2016.

175. NASA Visible Earth - Home. https://visibleearth.nasa.gov/collection/1484/blue-marble. Published October 7, 2019. Accessed October 7, 2019.

176. Hickendorff A, Bradt Travel Guides. *Gabon: The Bradt Travel Guide*.; 2014.

177. Auswärtiges Amt - Gabun. http://www.auswaertiges-amt.de/sid_95C2E68751E10988DFCFE3FFCA9177B8/DE/Aussenpolitik/Laender/Laenderinfos/01-Nodes_Uebersichtsseiten/Gabun_node.html. Accessed January 26, 2016.

178. Sylla EH, Lell B, Kun JF, Kremsner PG. Plasmodium falciparum transmission intensity and infection rates in children in Gabon. *Parasitol Res*. 2001;87(7):530-533.

179. Wildling E, Winkler S, Kremsner PG, Brandts C, Jenne L, Wernsdorfer WH. Malaria epidemiology in the province of Moyen Ogoov, Gabon. *Trop Med Parasitol Off Organ Dtsch Tropenmedizinische Ges Dtsch Ges Tech Zusammenarbeit GTZ*. 1995;46(2):77-82.

180. Dal-Bianco MP, Köster KB, Kombila UD, et al. High prevalence of asymptomatic Plasmodium falciparum infection in Gabonese adults. *Am J Trop Med Hyg*. 2007;77(5):939–942.

181. Sylla EHK, Kun JFJ, Kremsner PG. Mosquito distribution and entomological inoculation rates in three malaria-endemic areas in Gabon. *Trans R Soc Trop Med Hyg*. 2000;94(6):652-656. doi:10.1016/S0035-9203(00)90219-0

182. Woldearegai TG, Lalremruata A, Nguyen TT, et al. Characterization of Plasmodium infections among inhabitants of rural areas in Gabon. *Sci Rep*. 2019;9. doi:10.1038/s41598-019-46194-9

183. Nguetse CN, Adegnika AA, Agbenyega T, et al. Molecular markers of anti-malarial drug resistance in Central, West and East African children with severe malaria. *Malar J*. 2017;16. doi:10.1186/s12936-017-1868-y

184. Akue JP, Nkoghe D, Padilla C, et al. Epidemiology of Concomitant Infection Due to Loa loa and Mansonella perstans in Gabon. *PLOS Negl Trop Dis*. 2011;5(10):e1329. doi:10.1371/journal.pntd.0001329

185. Guideline IHT. Clinical safety data management: definitions and standards for expedited reporting. *Recomm Adopt Step*. 1994;4.

186. U.S. Department of Health and Human Services FDA Center for Biologics Evaluation and Research. Vaccines Guidances - Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. September 2007:10.

187. Rousset J-J, Association africaine de microbiologie et d'hygiène alimentaire. *Copro-parasitologie pratique: intérêt et méthodologie : notions sur les parasites du tube digestif = al-Kūbrūlūǰah al-ṭufaylīyah al-taṭbīqīyah*. Paris: Estem; 1993.

188. Weltgesundheitsorganisation, ed. *Manual of Basic Techniques for a Health Laboratory*. 2. ed. Geneva; 2003.

189. Planche T, Krishna S, Kombila M, et al. Comparison of methods for the rapid laboratory assessment of children with malaria. *Am J Trop Med Hyg*. 2001;65(5):599–602.

190. J. M. Goldsmid. Studies on the laboratory diagnosis of human filariasis: Preliminary communication. *J Clin Path*. 1970;(23):632-635.

191. Mathers C, Fat DM, Boerma JT, World Health Organization, eds. *The Global Burden of Disease: 2004 Update*. Geneva, Switzerland: World Health Organization; 2008.

192. Sachs J, Malaney P. The economic and social burden of malaria. *Nature*. 2002;415(6872):680-685. doi:10.1038/415680a

193. World Health Organization, World Health Organization, Global Malaria Programme. *Global Technical Strategy for Malaria, 2016-2030*. Geneva, Switzerland; 2015.

194. Beeson JG, Drew DR, Boyle MJ, Feng G, Fowkes FJI, Richards JS. Merozoite surface proteins in red blood cell invasion, immunity and vaccines against malaria. *FEMS Microbiol Rev*. 2016;40(3):343-372. doi:10.1093/femsre/fuw001

195. Sheehy SH, Duncan CJA, Elias SC, et al. ChAd63-MVA-vectored blood-stage malaria vaccines targeting MSP1 and AMA1: assessment of efficacy against mosquito bite challenge in humans. *Mol Ther J Am Soc Gene Ther*. 2012;20(12):2355-2368. doi:10.1038/mt.2012.223

196. Sirima SB, Tiono AB, Ouédraogo A, et al. Safety and Immunogenicity of the Malaria Vaccine Candidate MSP3 Long Synthetic Peptide in 12–24 Months-Old Burkinabe Children. *PLOS ONE*. 2009;4(10):e7549. doi:10.1371/journal.pone.0007549

197. Davidsen J, Rosenkrands I, Christensen D, et al. Characterization of cationic liposomes based on dimethyldioctadecylammonium and synthetic cord factor from M. tuberculosis (trehalose 6,6'-dibehenate)-a novel adjuvant inducing both strong CMI and antibody responses. *Biochim Biophys Acta*. 2005;1718(1-2):22-31. doi:10.1016/j.bbamem.2005.10.011

198. Mordmüller B, Supan C, Sim KL, et al. Direct venous inoculation of Plasmodium falciparum sporozoites for controlled human malaria infection: a dose-finding trial in two centres. *Malar J*. 2015;14:117. doi:10.1186/s12936-015-0628-0

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

200. Moorthy VS, Diggs C, Ferro S, et al. Report of a Consultation on the Optimization of Clinical Challenge Trials for Evaluation of Candidate Blood Stage Malaria Vaccines, 18–19 March 2009, Bethesda, MD, USA. *Vaccine*. 2009;27(42):5719-5725. doi:10.1016/j.vaccine.2009.07.049

201. Bethony JM, Simon G, Diemert DJ, et al. Randomized, placebo-controlled, double-blind trial of the Na-ASP-2 Hookworm Vaccine in unexposed adults. *Vaccine*. 2008;26(19):2408-2417. doi:10.1016/j.vaccine.2008.02.049

202. Diemert DJ, Pinto AG, Freire J, et al. Generalized urticaria induced by the Na-ASP-2 hookworm vaccine: Implications for the development of vaccines against helminths. *J Allergy Clin Immunol*. 2012;130(1):169-176.e6. doi:10.1016/j.jaci.2012.04.027

203. Stanisic DI, McCarthy JS, Good MF. Controlled Human Malaria Infection: Applications, Advances, and Challenges. *Infect Immun*. 2018;86(1). doi:10.1128/IAI.00479-17

204. McCall MBB, Kremsner PG, Mordmüller B. Correlating efficacy and immunogenicity in malaria vaccine trials. *Semin Immunol*. 2018;39:52-64. doi:10.1016/j.smim.2018.08.002

205. Epstein JE, Paolino KM, Richie TL, et al. Protection against Plasmodium falciparum malaria by PfSPZ Vaccine. *JCI Insight*. 2016;2(1):e89154. doi:10.1172/jci.insight.89154

206. Sissoko MS, Healy SA, Katile A, et al. Safety and efficacy of PfSPZ Vaccine against Plasmodium falciparum via direct venous inoculation in healthy malaria-exposed adults in Mali: a randomised, double-blind phase 1 trial. *Lancet Infect Dis*. 2017;17(5):498-509. doi:10.1016/S1473-3099(17)30104-4

207. Karlsson I, Brandt L, Vinner L, et al. Adjuvanted HLA-supertype restricted subdominant peptides induce new T-cell immunity during untreated HIV-1-infection. *Clin Immunol*. 2013;146(2):120-130. doi:10.1016/j.clim.2012.12.005

208. Zhang L, Wang W, Wang S. Effect of Vaccine Administration Modality on Immunogenicity and Efficacy. *Expert Rev Vaccines*. 2015;14(11):1509-1523. doi:10.1586/14760584.2015.1081067

209. Martin BL, Nelson MR, Hershey JN, Engler RJM. Adverse reactions to vaccines. *Clin Rev Allergy Immunol*. 2003;24(3):263-275. doi:10.1385/CRIAI:24:3:263

210. Hermsen CC, Verhage DF, Telgt DSC, et al. Glutamate-rich protein (GLURP) induces antibodies that inhibit in vitro growth of Plasmodium falciparum in a phase 1 malaria vaccine trial. *Vaccine*. 2007;25(15):2930-2940. doi:10.1016/j.vaccine.2006.06.081

211. WHO. Information sheet observed rates of vaccine reactions Hepatitis B vaccine. June 2012. https://www.who.int/vaccine_safety/initiative/tools/Hep_B_Vaccine_rates_information_sheet.pdf . Accessed July 26, 2019.

212. Muturi-Kioi V, Lewis D, Launay O, et al. Neutropenia as an Adverse Event following Vaccination: Results from Randomized Clinical Trials in Healthy Adults and Systematic Review. *PLoS ONE*. 2016;11(8). doi:10.1371/journal.pone.0157385

213. Regules JA, Cummings JF, Ockenhouse CF. The RTS,S vaccine candidate for malaria. *Expert Rev Vaccines*. 2011;10(5):589-599. doi:10.1586/erv.11.57

214. Mata E, Salvador A, Igartua M, Hernández RM, Pedraz JL. Malaria Vaccine Adjuvants: Latest Update and Challenges in Preclinical and Clinical Research. *BioMed Res Int*. 2013;2013. doi:10.1155/2013/282913

215. Bart Marco Tamborrini, Sabine A Stoffel. Immunogenicity of a virosomally-formulated Plasmodium falciparum GLURP-MSP3 chimeric protein-based malaria vaccine candidate in comparison to adjuvanted formulations. *Malar J*. December 2011.

216. Kammer AR, Amacker M, Rasi S, et al. A new and versatile virosomal antigen delivery system to induce cellular and humoral immune responses. *Vaccine*. 2007;25(41):7065-7074. doi:10.1016/j.vaccine.2007.07.052

217. Baldwin SL, Roeffen W, Singh SK, et al. Synthetic TLR4 agonists enhance functional antibodies and CD4+ T-cell responses against the Plasmodium falciparum GMZ2.6C multi-stage vaccine antigen. *Vaccine*. 2016;34(19):2207-2215. doi:10.1016/j.vaccine.2016.03.016

218. Miura K. Progress and prospects for blood-stage malaria vaccines. *Expert Rev Vaccines*. 2016;15(6):765-781. doi:10.1586/14760584.2016.1141680

219. Adegnika AA, Ramharter M, Agnandji ST, et al. Epidemiology of parasitic co-infections during pregnancy in Lambaréné, Gabon: Pregnancy and parasitic co-infection. *Trop Med Int Health*. 2010;15(10):1204-1209. doi:10.1111/j.1365-3156.2010.02598.x

220. Girgis NM, Gundra UM, Loke P. Immune Regulation during Helminth Infections. *PLoS Pathog*. 2013;9(4). doi:10.1371/journal.ppat.1003250

221. Sabin EA, Araujo MI, Carvalho EM, Pearce EJ. Impairment of tetanus toxoid-specific Th1-like immune responses in humans infected with Schistosoma mansoni. *J Infect Dis*. 1996;173(1):269-272. doi:10.1093/infdis/173.1.269

222. Riner DK, Ndombi EM, Carter JM, et al. Schistosoma mansoni Infection Can Jeopardize the Duration of Protective Levels of Antibody Responses to Immunizations against Hepatitis B and Tetanus Toxoid. *PLoS Negl Trop Dis*. 2016;10(12):e0005180. doi:10.1371/journal.pntd.0005180

223. Wajja A, Kizito D, Nassanga B, et al. The effect of current Schistosoma mansoni infection on the immunogenicity of a candidate TB vaccine, MVA85A, in BCG-vaccinated adolescents: An open-label trial. *PLoS Negl Trop Dis*. 2017;11(5). doi:10.1371/journal.pntd.0005440

224. Ondigo BN, Muok EMO, Oguso JK, et al. Impact of Mothers' Schistosomiasis Status During Gestation on Children's IgG Antibody Responses to Routine Vaccines 2 Years Later and Anti-Schistosome and Anti-Malarial Responses by Neonates in Western Kenya. *Front Immunol*. 2018;9. doi:10.3389/fimmu.2018.01402

225. Cooper PJ, Chico M, Sandoval C, et al. Human Infection with Ascaris lumbricoides Is Associated with Suppression of the Interleukin-2 Response to Recombinant Cholera Toxin B Subunit following Vaccination with the Live Oral Cholera Vaccine CVD 103-HgR. *Infect Immun*. 2001;69(3):1574-1580. doi:10.1128/IAI.69.3.1574-1580.2001

226. Brown J, Baisley K, Kavishe B, et al. Impact of malaria and helminth infections on immunogenicity of the human papillomavirus-16/18 AS04-adjuvanted vaccine in Tanzania. *Vaccine*. 2014;32(5):611-617. doi:10.1016/j.vaccine.2013.11.061

227. Nakalembe M, Banura C, Namujju PB, Mirembe FM. Immunogenicity to the bivalent HPV-16/18 vaccine among adolescent African students exposed to helminths and malaria. *J Infect Dev Ctries*. 2015;9(2):197-205. doi:10.3855/jidc.5719

228. McKittrick ND, Malhotra IJ, Vu DM, et al. Parasitic infections during pregnancy need not affect infant antibody responses to early vaccination against Streptococcus pneumoniae, diphtheria, or Haemophilus influenzae type B. *PLoS Negl Trop Dis*. 2019;13(2):e0007172. doi:10.1371/journal.pntd.0007172

229. Ayash-Rashkovsky M, Weisman Z, Diveley J, Moss RB, Bentwich Z, Borkow G. Generation of Th1 immune responses to inactivated, gp120-depleted HIV-1 in mice with a dominant Th2 biased immune profile via imunostimulatory oligonucleotides—relevance to AIDS vaccines in developing countries. *Vaccine*. 2002;20(21):2684-2692. doi:10.1016/S0264-410X(02)00202-5

230. Galvani AP. AGE-DEPENDENT EPIDEMIOLOGICAL PATTERNS AND STRAIN DIVERSITY IN HELMINTH PARASITES. *J Parasitol*. 2005;91(1):24-30. doi:10.1645/GE-191R1

231. Chuangchaiya S, Persson KEM. How Should Antibodies against P. falciparum Merozoite Antigens Be Measured? *J Trop Med*. 2013;2013. doi:10.1155/2013/493834

7. Personal contributions

The clinical trial, on which the current thesis is based, was a collaborative work of the Institute for Tropical Medicine Tübingen and the *Centre de Recherches Médicales de Lambaréné* (CERMEL).

Responsible for the concept and conduction of the study were Prof. Dr. med. Benjamin Mordmüller, Dr. Ulysse Ateba Ngoa, and Prof. Dr. med. Ayola Akim Adegnika. Andreas Homoet assisted in the planning of the trial by creation of study documents such as patient and case report forms. The publication was written by all authors under the supervision of Prof. Dr. med. Benjamin Mordmüller.

Recruiting of participants, tolerability assessment during the follow up such as AE directed interviews, basic clinical examinations, and reporting of AEs was done by Andreas Homoet, Dr. Ulysse Ateba Ngoa, Dr. Jean Ronald Edoa, and Dr. Jean Claude Dejon-Agobe.

Immunological assays were mainly performed by Odilon Paterne Nouatin (ELISA). Assessment of helminth infection was done by the parasitology routine laboratory (Urine filtration, copro culture, Kato Katz method, and modified Knott`s technique). Andreas Homoet assisted during these procedures.

Statistical analysis for this thesis was performed by Andreas Homoet.

The thesis was written alone by Andreas Homoet with the support of Dr. Ulysse Ateba Ngoa and the doctoral supervisor Prof. Dr. med. Benjamin Mordmüller.

8. Publications

Parts of this thesis have already been published in the following papers:

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 Controlled Human Malaria Infection of Healthy Adults With Lifelong Malaria Exposure to Assess Safety, Immunogenicity, and Efficacy of the Asexual Blood-stage Malaria Vaccine Candidate GMZ2. Clin. Infect. Dis. (2018). doi:10.1093/cid/ciy1087

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And finally, I want to express my gratitude towards my two sisters and my parents for their love, faith and support during my studies, writing of this thesis and throughout my whole life.

10. Curriculum vitae

Date of Birth: 05th November 1989

Nationality: German

Educational career:

1996 – 2000: Willibrordschule, Steinfurt

2000 – 2009: Gymnasium Arnoldinum, Steinfurt

Civil Service:

2009 – 2010: Medical Centre Rheine

Academic career:

Apr. 2011 – Nov. 2018	Medical studies, Eberhard Karls University, Tübingen
19th March 2013	First medical examination
12th October 2017	Second medical examination
30th November 2018	Third medical examination
Apr. 2015 – Feb. 2016	Research stay in Lambaréné, Gabon.
2015 – 2020	Doctoral Thesis

Practical experiences:

Jan. 2019 – Dec. 2019	Occupational medicine

11. Appendix:

Table 11: *Toxicity scale for laboratory values, adapted from FDA Center for Biologics Evaluation and Research. Vaccines Guidances - Guidance for Industry*[186]

Parameter	**Unit**	**Ref. interval**		**Severity of abnormality**			
		lower	upper	Grade 1	Grade 2	Grade 3	Grade 4
HGB	g/dL	12	16	10.5	8.5	6.5	<6.5
Leucocytes	/nL	4	9.5	15	20	25	>25.0
				2.5	1.5	1	<1.0
Thrombocytes	/nL	150	450	125	100	25	<25
Neutrophils	/nL	1.6	7.6	1.3	1	0.5	<0.5
Eosinophils	/nL	0.04	4.9	9	15	>15	"hyper-eosinophilia"
Lymphocytes	/nL	0.8	4.3	0.65	0.5	0.25	<0.25
Creatinine	mg/dL		1.1	1.7	2	2.5	>2.5
ALT	U/L		50	125	255	500	>10
AST	U/L		50	125	255	500	>10

Table 12: *Composition of buffers for the ELISA*

1-liter washing buffer	
2 PBS tablets	Gibco cat # 18912-014
1ml Tween 20	TMB ONE, KEM EN TEC cat # 4380
29 gr. NaCL	Merck cat # 1.06404.1000
1 l. H_2O	
1-liter blocking buffer	
2 PBS tablets	Gibco cat # 18912-014
1ml Tween 20	TMB ONE, KEM EN TEC cat # 4380
30 gr. milk powder	Merck cat # 1.06404.1000
1 l. H_2O	
1-liter dilution buffer	
2 PBS tablets	Gibco cat # 18912-014
1ml Tween 20	TMB ONE, KEM EN TEC cat # 4380
10 gr. milk powder	Merck cat # 1.06404.1000
1 l. H_2O	

Table 13: *List of materials*

ELISA	
Tween 20	Sigma, USA
TMB ONE	KEM EN TEC, Denmark
Phosphate buffered saline tablets	Thermo Fisher Scientific (Gibco), USA
Naturaflor ®, dry skimmed non-fat milk	Töpfer, Dietmannsried
NaCl	Merck, Darmstadt
Peroxidase conjugated goat anti-human IgG	Thermo Fisher Scientific (Invitrogen), USA
Purified human polyclonal IgG (Standard)	Binding site (is not available anymore)
Positive serum samples	Serum pool of semi-immune participants of former GMZ2 trials
Negative serum samples	Malaria naïve European blood samples
GMZ2 final concentration 0.5µg/ml GLURP final concentration 0.5µg/ml MSP3 final concentration 1µg/ml	Michael Theisen, Denmark
Nunc-Immuno 96 MicroWell solid plates	Merck, Darmstadt
Corning® Multipette® 12-pette	Merck, Darmstadt
H_2SO_4	Thermo Fisher Scientific (Invitrogen), USA
Anti-human IgG, 0.5µg/ml	Sigma, USA
Parasitological assessment	
Whatman12µm (Ø 25 mm, 12µm pore size)	Merck, Darmstadt
Laboratory kit for the Kato-Katz method	Sterlitech Cooperation, USA
Sieve	Screen, stainless steel 60-125 mesh
3% Malachite green oxalate solution	Merck, Darmstadt
Template of 41.7mg	Vestergaard Frandsen SA, Aarhus, Denmark
TBS	
Giemsa stain, pH 6.9solution	Sigma, USA

Adverse events and their correlation to elicited Antibodies

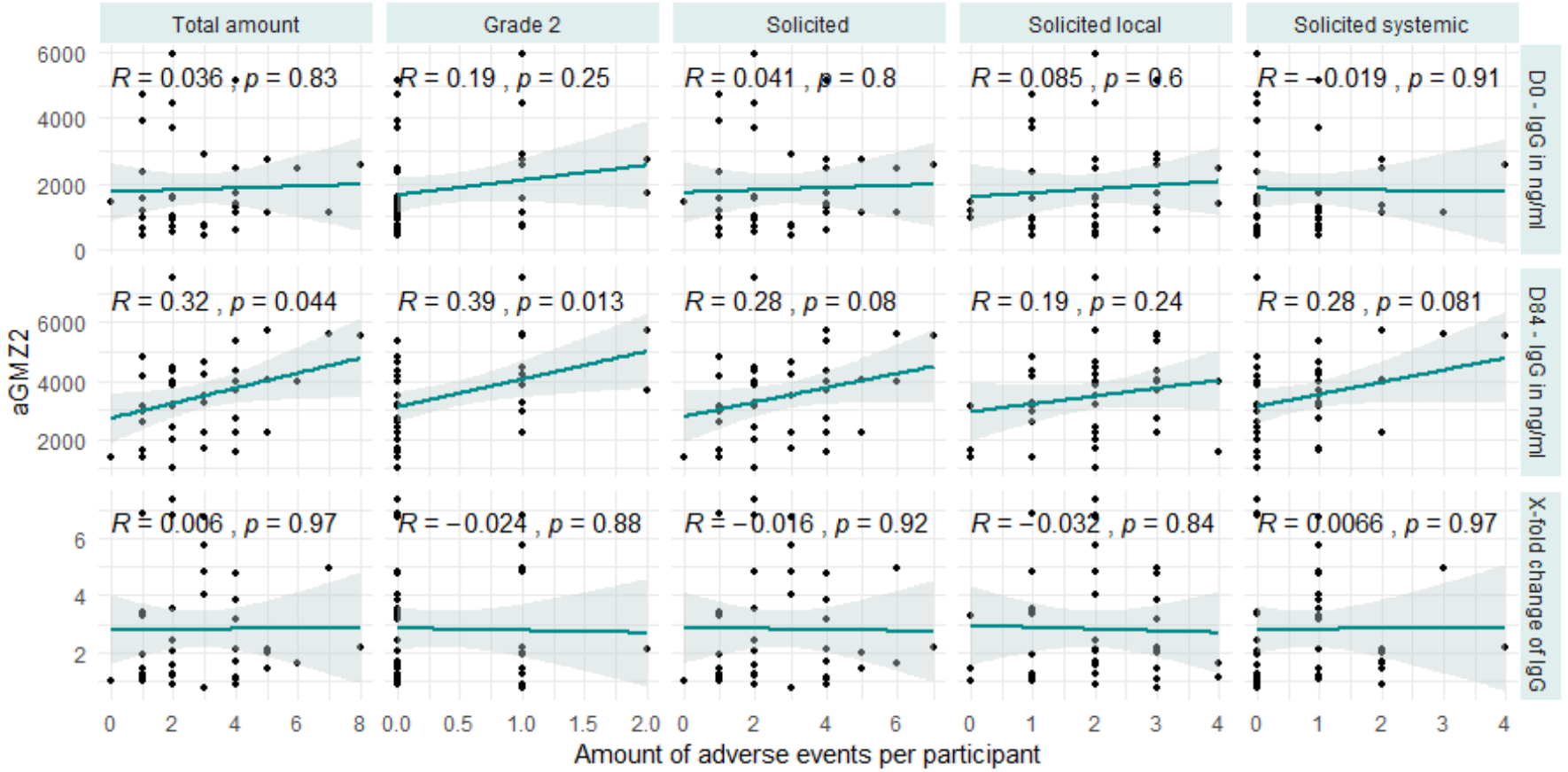

Figure 34: *Correlation between AEs and antibodies against GMZ2.*

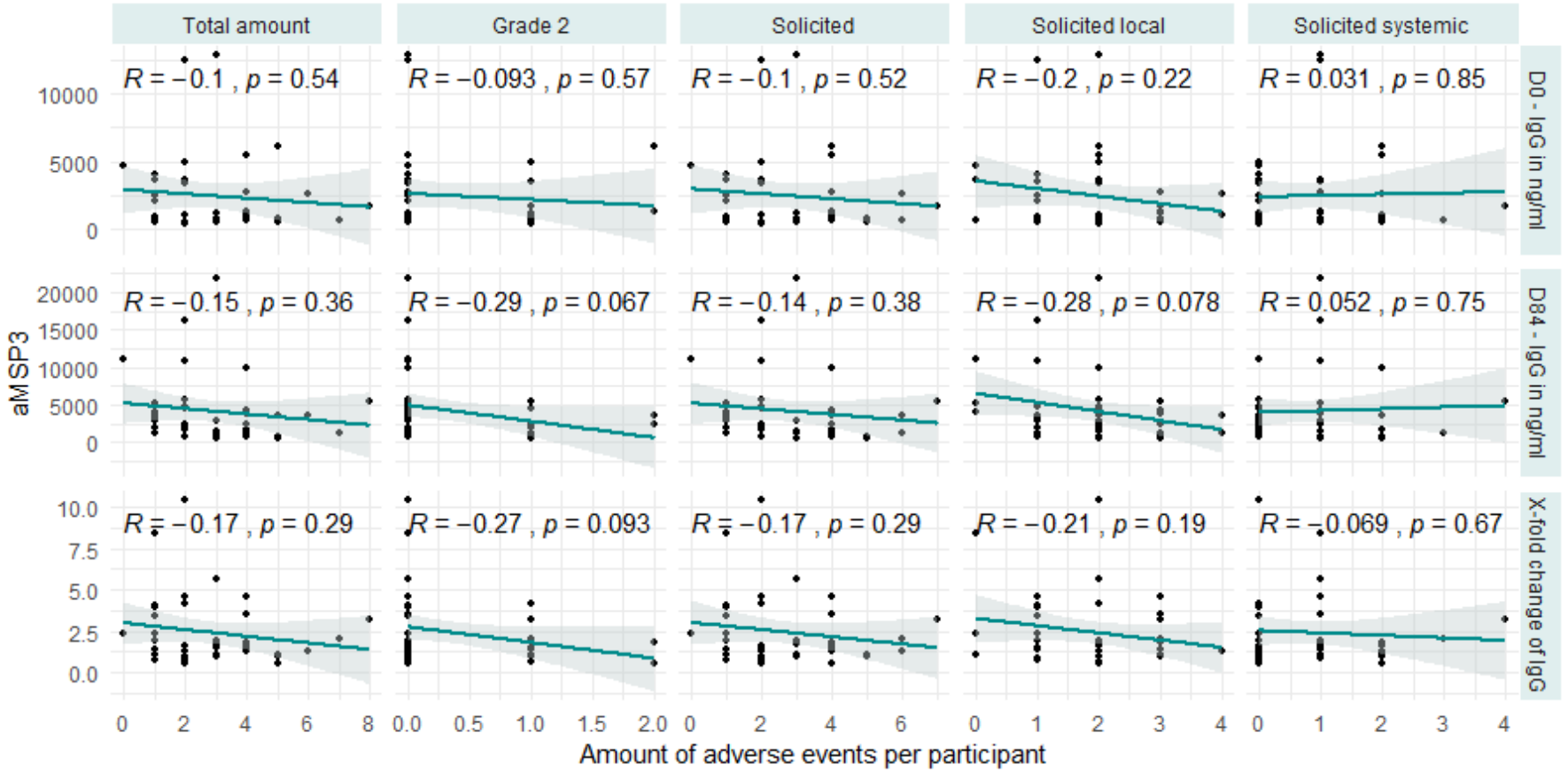

Figure 35: *Correlation between AEs and antibodies against MSP3*

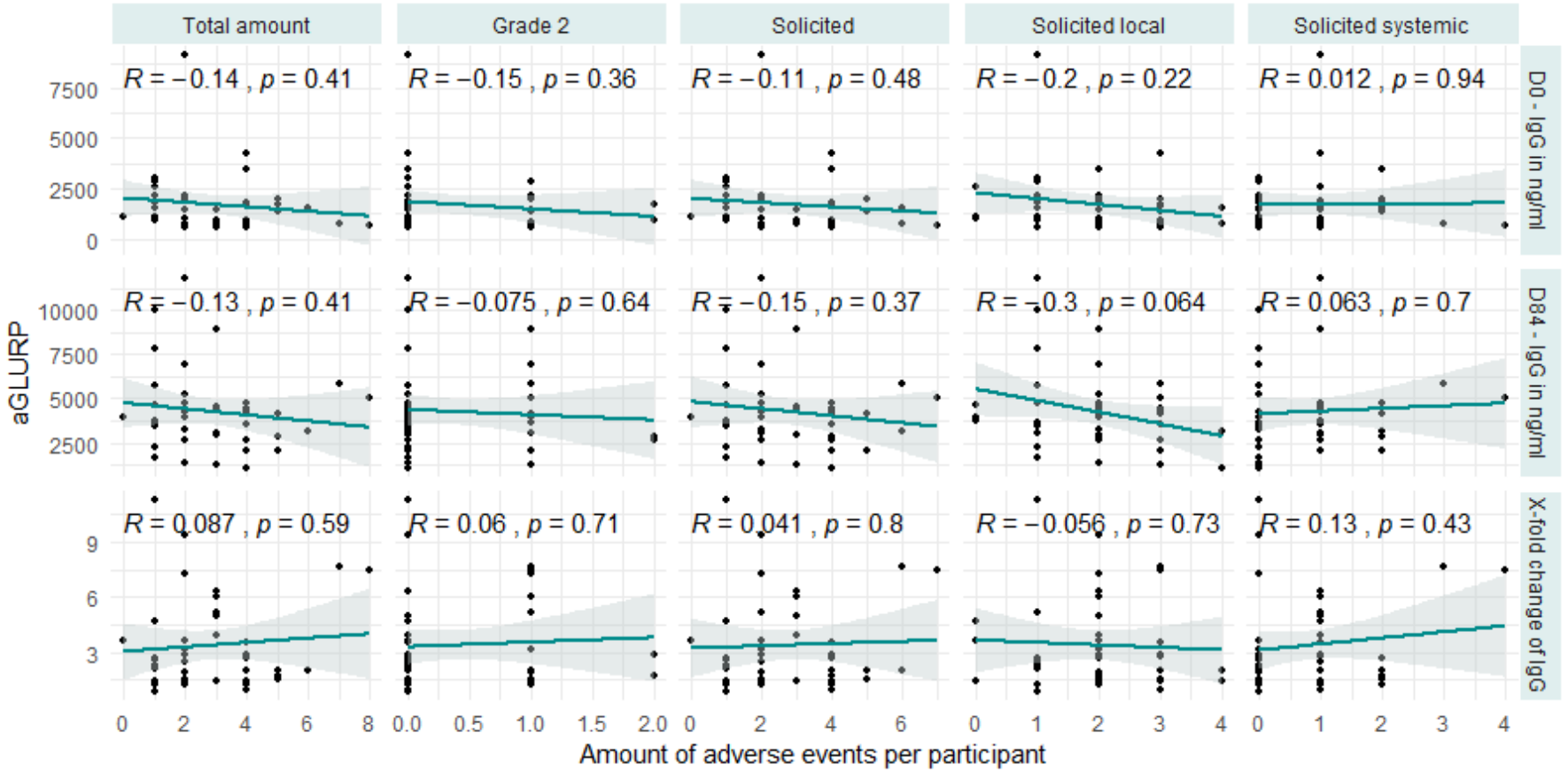

Figure 36: *Correlation between AEs and antibodies against GLURP.*

Laboratory values and their correlation to elicited antibodies

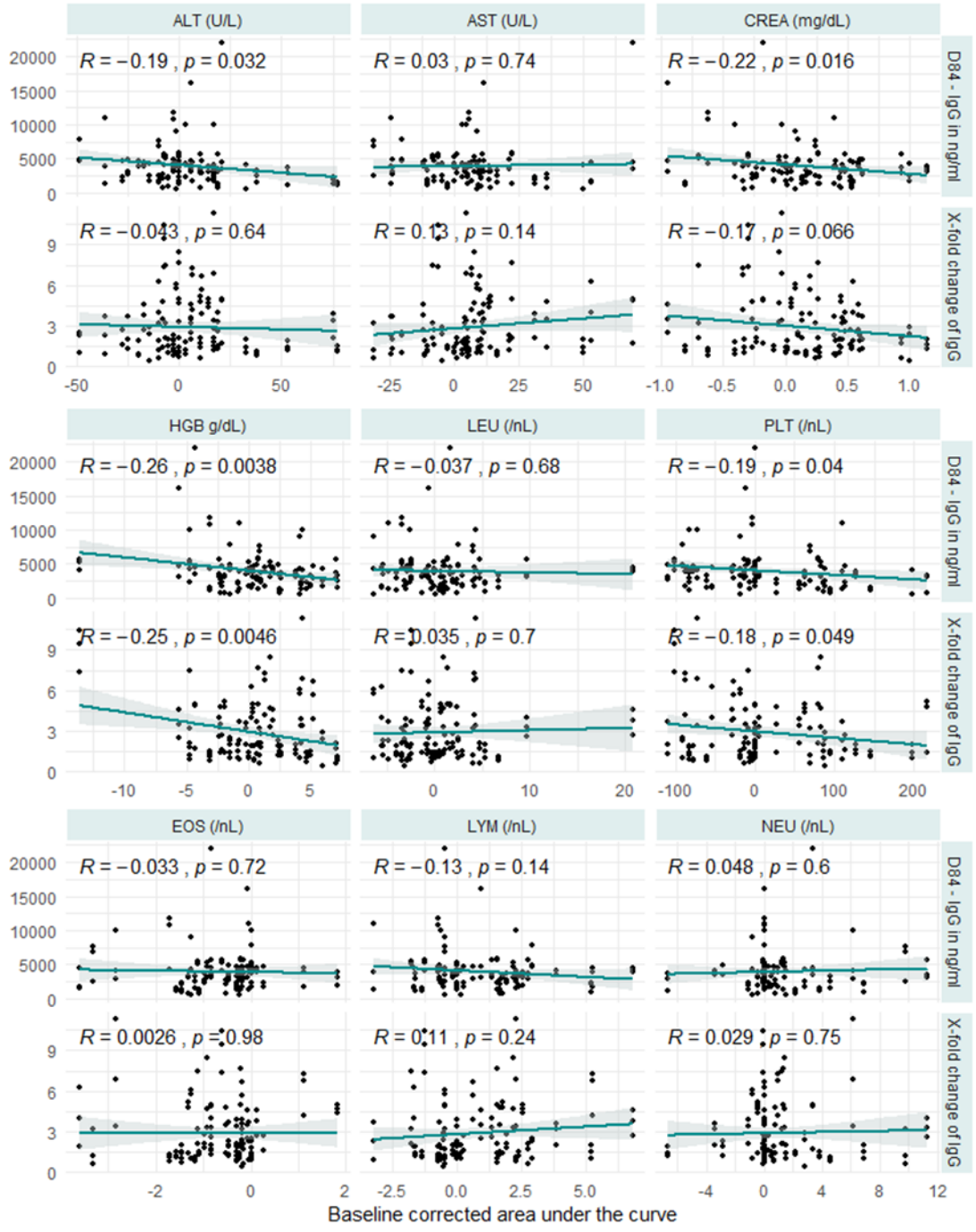

Figure 37: *Correlation between trends of laboratory values and vaccine induced immunogenicity.*

Vaccine specific antibodies of helminth infection groups D0 and D84

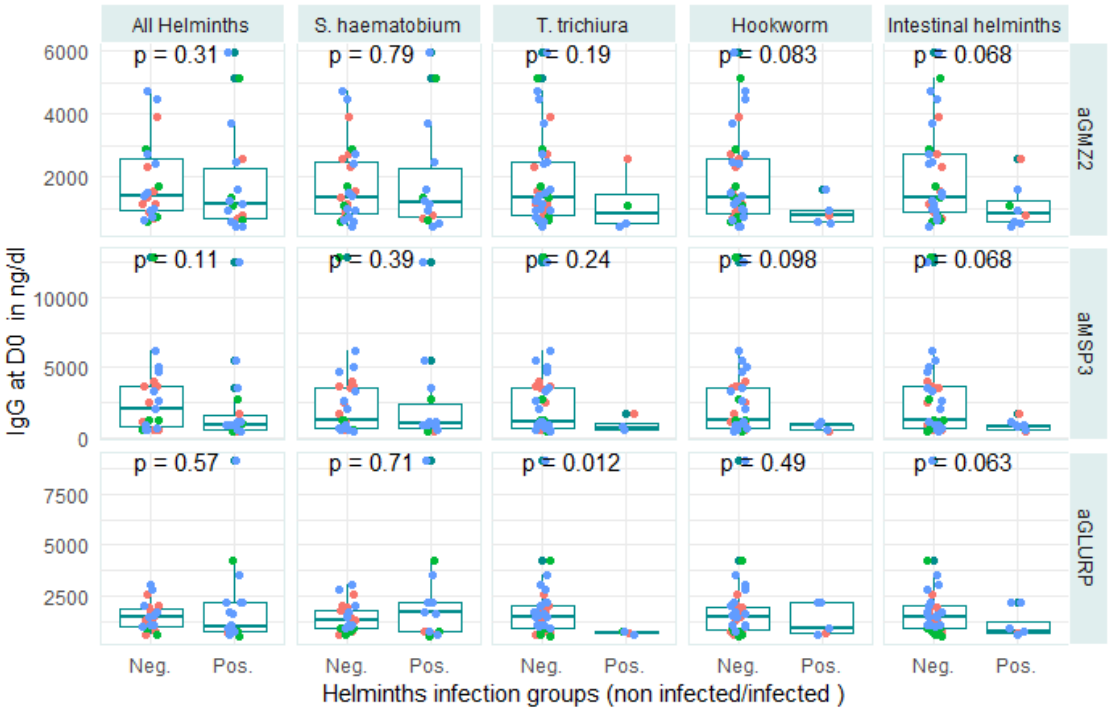

Figure 38: *Comparison of the amount of IgG against GMZ2, MSP3 and GLURP at day 0. The colors of the dots display the vaccination groups: Red = GMZ2-Alhydrogel, green = 30µg of GMZ2-CAF01, blue = 100µg of GMZ2-CAF01.*

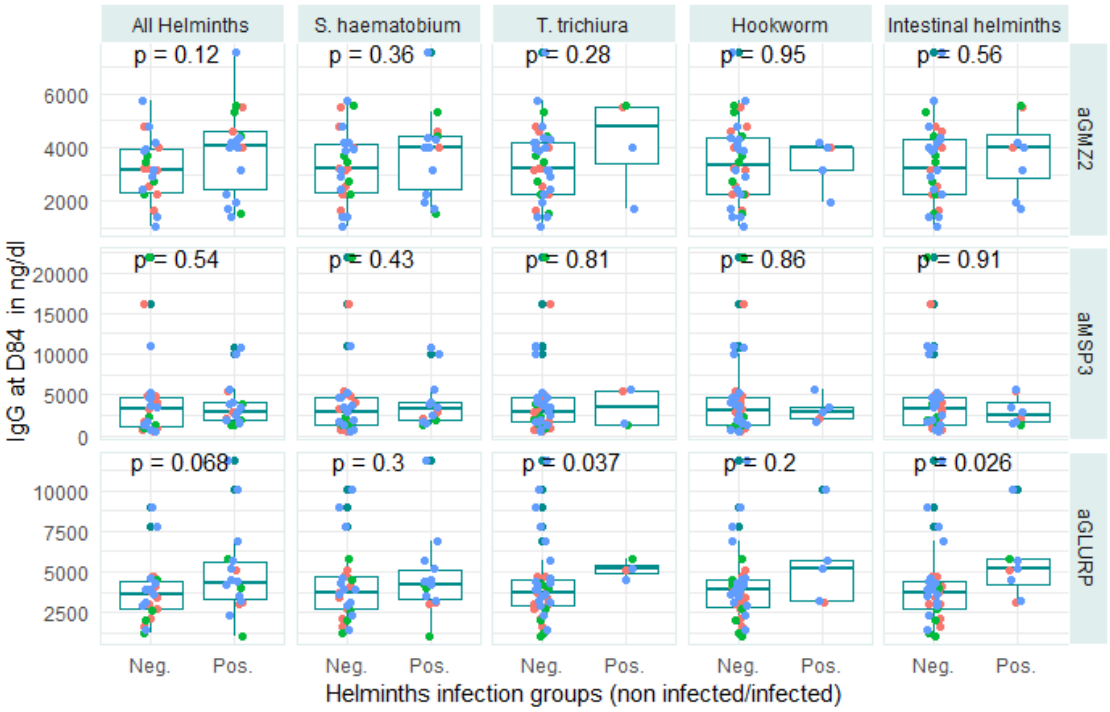

Figure 39: *Comparison of the amount of IgG against GMZ2, MSP3 and GLURP at day 84. The colors of the dots display the vaccination groups: Red = GMZ2-Alhydrogel, green = 30µg of GMZ2-CAF01, blue = 100µg of GMZ2-CAF01.*