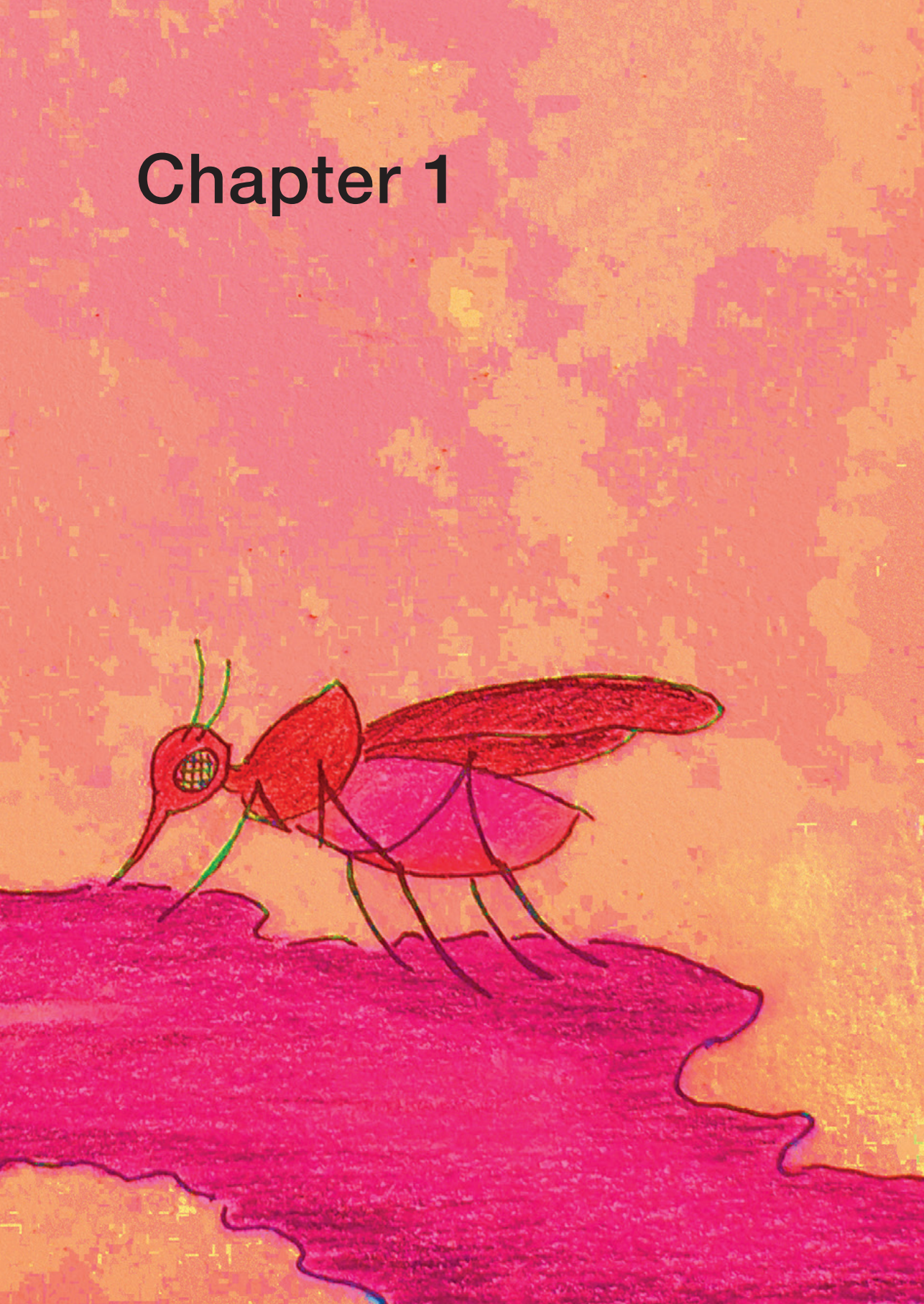
Malaria vaccinology: from the development of a new malaria vaccine to the ethics of clinical trial participation

Lamers, O.A.C.

Chapter 1



Introduction

Malaria

What is malaria?

Malaria is a vector-borne disease caused by the protozoan parasite *Plasmodium* and transmitted by female *Anopheles* mosquitoes. There are five species of *Plasmodia* that can cause disease in humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi*. *Plasmodium falciparum* (*Pf*) is by far the most common and virulent species[1] and will be the focus of this thesis.

Malaria life cycle and symptomatology

Sporozoites are injected into humans by female *Anopheles* mosquitoes during a blood meal and then travel to the liver via the circulation. Schizonts develop inside infected hepatocytes and release merozoites into the blood. Merozoites then infect erythrocytes, multiply asexually and eventually cause their host cells to burst, perpetuating the cycle of re-infection[2]. Gametogenesis, that is the production of sexual forms of the parasite, occurs in response to environmental stress in the host. Gametocytes are taken up by *Anopheles* mosquitoes during a blood meal and develop into ookinetes and then oocysts in the mosquito midgut and finally into sporozoites in the salivary glands[3,4]. From here, the life-cycle begins anew (**Fig. 1**). Even though the pre-erythrocytic stage of the *Pf* life cycle is asymptomatic, immunologically it is anything but silent: liver-resident cellular immune responses are thought to contribute to protection against the disease[5]. The cyclic rupture of erythrocytes causes a strong inflammatory response and the typical symptoms of malaria including fever and malaise. Serious complications, such as cerebral malaria and multiorgan failure, may arise in response to mechanical sequestration of infected erythrocytes to capillaries and widespread inflammation[1,6].

Malaria epidemiology and control

In 2023 alone malaria caused 263 million cases of disease and 597.000 deaths. The WHO African region is disproportionally affected, the most vulnerable populations being children under the age of five and pregnant women[7]. While malaria incidence had been steadily declining since 2000, it experienced a resurgence in 2019, most likely due to the negative effects of the SARS-CoV-2 pandemic on preventative strategies[8].

Resistance against antimalarial drugs is another factor that may compromise efficacy of both clinical and preventative treatment. Resistance to artemisinin, the most efficacious antimalarial drug currently available, is especially worrisome: first reported in Asia in

2008[9], it has since spread to the African continent as well. It is caused by several different mutations in the *k13* gene, which codes for a putative E3 ligase adaptor[10,11] and results in delayed clearance of parasitemia. The projection is that, combined with resistance to partner drugs, decreased sensitivity to artemisinin will eventually lead to treatment failure[11].

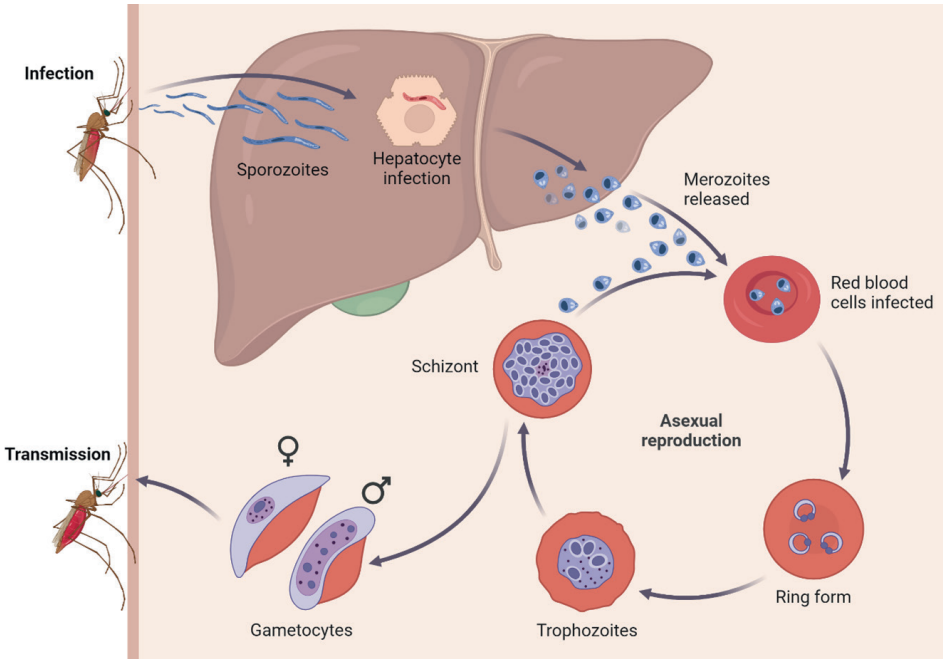


Figure 1. *Plasmodium* life-cycle
Plasmodium enters its human host when a mosquito takes a blood meal. Sporozoites move through the skin, into the circulation and then into the liver. Schizonts are formed inside infected hepatocytes which, upon exponential replication, release merozoites into the blood. Merozoites grow and multiply asexually inside erythrocytes until the host cells burst, releasing even more merozoites. Gametocytes are the sexual forms of the parasite and can be picked up by a mosquito during a new blood meal. In the mosquito gut, gametocytes form ookinetes and oocysts, which hatch sporozoites. Sporozoites then migrate to salivary glands from where they are injected into their human hosts during the mosquito's next blood meal.

Historically, vector control has been one of the most effective ways of eradicating and controlling malaria. After the ban of dichlorodiphenyltrichloroethane (DDT) due to environmental and health concerns, insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) remain the two most important tools for vector control[12]. ITNs serve the dual function of physical barrier against biting and of chemical repellent killing mosquitoes that come in contact with them. IRS is very similar to ITNs with the only difference that insecticides are applied directly to indoor surfaces[13]. However, resistance

to insecticides is increasing and, in combination with climate change, may compromise established control over vector populations[14,15].

Hence, the rising resistance of both parasites against drugs and mosquitoes against insecticides risk to make pharmacological and chemical interventions obsolete in the not-so-distant future[9,16-18]. If the ambitious goal set forward by the WHO Global Technical Strategy[19] of reducing malaria mortality by 90% in 2030 is to be met, new and more effective tools of eradication are needed. Availability of an effective vaccine could greatly reduce the incidence and transmission of malaria and be the decisive factor finally leading to disease eradication[20,21].

Natural immunity to malaria

No such thing as sterile immunity to malaria exists in nature, as continuous re-exposure is required for the development of both tolerance against severe symptoms and resistance to parasites[22].

Natural protection against malaria is thought to rely on humoral immune responses against erythrocytic antigens, as neither parasites nor erythrocytes have HLA class I or II molecules that could be recognized by T cells[23,24]. The specificity of the antibody response is limited by the strong pro-inflammatory environment of the blood-stage infection, which impairs germinal centre formation and effective B cell maturation through excessive interferon-γ (IFN-γ) production[23,25]. Antibodies that protect against severe malaria, such as those directed at the erythrocyte membrane protein 1 (PfEMP1), develop early after the first exposure while prevention of symptoms and inhibition of erythrocyte invasion ensues later in life with increasing titres and broadening of the immune response[23].

However, despite clinical tolerance, infected individuals may still carry asexual and sexual forms of the parasite, thereby perpetuating the cycle of transmission[22].

Vaccine induced immunity to malaria

In contrast to naturally acquired immunity, which elicits a broad immune response, most vaccines are designed to generate a limited yet highly specific response. Ideally, any vaccine against malaria would not only mitigate symptoms, but also prevent their onset and block transmission. It is for this reason that much research has been directed at inducing pre-erythrocytic immunity, which could not only stop merozoites egression from the liver, but also formation of gametocytes[26]. Hence, this is where the focus of this thesis will lie.

Mechanisms of vaccine-induced immunity

The mechanisms underlying vaccine-induced pre-erythrocytic immunity are complex and incompletely understood.

Cellular immunity

Cellular immune responses play a central role in protection against the liver stages of the parasite. Immune patrolling by T cells is dependent on cell recirculation in lymphoid tissues (central effector (T_{CM}) cells that are CCR7$^+$), in non-lymphoid tissues (effector cells (T_{EM}) that are CCR7$^-$), and by tissue residency (characterized by the expression of CD103$^+$)[27].

In malaria, CD8$^+$ T cells are thought to be primed in the lymph nodes by dendritic cells (DCs) and then migrate to the liver where they become tissue-resident effector (CCR7$^-$ CD103$^+$ CD8$^+$) cells and directly kill infected hepatocytes through perforin and granzyme. A proportion of these T cells may then acquire a memory phenotype and persist even after infection clearance[28,29].

CD4$^+$ T cells are another subset that has been intensively studied in malaria. They contribute to CD8$^+$ T cell activation and persistent proliferation by promoting antigen presentation and suppressing apoptosis[30]. Additionally, CD4$^+$ T cells are thought to have a direct anti-parasitic effect through the production of pro-inflammatory cytokines and cytotoxic granules, possibly even killing infected hepatocytes[31].

Humoral immunity

While antibodies are an important component of naturally acquired immunity, their role in pre-erythrocytic immunity is unclear[32]. Most research has focussed on the circumsporozoite protein (CSP), as this is the major protein on the sporozoite surface. Subunit vaccines, designed to induce a strong humoral immune response against CSP, have limited efficacy which diminishes further with decreasing antibody titres[33]. The use of anti-CSP antibodies as correlates of protection also has limited value[34-37]. While it has been shown that sufficient amounts of monoclonal antibodies with high affinity for CSP can protect against infection[38,39], both the quality and quantity of antibodies produced after immunization does not always reach the protective threshold[34-37].

Pre-erythrocytic malaria vaccine candidates

The quest for developing an effective vaccine against malaria has been ongoing for decades, hampered by the complex biology of *Plasmodium* and by a relative scarcity

of funds[22]. There are currently two types of pre-erythrocytic vaccine candidates, namely subunit vaccines and vaccines consisting of live, attenuated parasites[26]. The efficacy of such vaccines can be tested by controlled human malaria infection (CHMI) studies, where healthy participants are exposed to live unattenuated parasites in a controlled setting[40].

Subunit vaccines

The first licensed malaria vaccine was RTS,S/A01 (marketed as Mosquirix), a subunit vaccine based on CSP, in combination with hepatitis B surface antigen and the AS01 adjuvant. It was developed by scientists at GlaxoSmithKline (GSK) and the Walter Reed Army Institute and has recently been approved by the WHO for widespread administration in malaria-endemic countries: protection varies between 30% and 50% in infants and children and wanes over time[41-43]. Similar results were reported for adults[44].

Attempts at improving the efficacy of RTS,S have brough forward R21, yet another subunit vaccine consisting of a virus-like particle containing CSP fused to hepatitis B surface antigen and formulated with the adjuvant Martrix-M. In a recent trial conducted across four African sites and involving children aged 5-36 months, vaccine efficacy amounted to between 68% and 75% after one year[45]. However, despite these promising results, there is currently insufficient evidence to conclude that, on the long term, the efficacy of R21 will be superior to that of RTS,S/A01[46].

Live attenuated vaccines

An alternative to subunit vaccines are whole SPZ (WSp) vaccines, which have shown up to 100% efficacy in malaria naïve populations. WSp vaccines are live malaria parasites, attenuated by radiation, pharmacological intervention or genetic modification[47,48].

The first experiments with radiation attenuated sporozoites (RAS) date back to the sixties of the previous century: after protection had been achieved in mice [49], findings were quickly translated to humans[50-52]. RAS can be administered either by mosquito bites or as an injectable product (PfSPZ Vaccine). Irradiated sporozoites reach the liver, but arrest development after 24 to 48 hours. While RAS are safe and well-tolerated, protection requires high doses (up to 2000 mosquito bites) in malaria-naïve individuals[48] and in pre-exposed populations protection remains low despite increasing doses[53]. One study in Tanzania found that administration of cryopreserved sporozoites in excess of 9×10^5 was actually associated with a decrease in protection[54].

Another possibility is the administration of live sporozoites in combination with chemoprophylaxis (CPS). This has, for a long time, been the most efficient WSp approach,

as three immunizations with a total of 45 mosquito bites were sufficient to induce sterile immunity in malaria-naïve participants[55]. The superior efficacy of this method has been attributed to the prolonged exposure and increased antigenic load presented to the immune system. However, CPS is less suitable for large-scale implementation because it bears the intrinsic risk of un-attenuated parasite administration to healthy individuals.

More recently, genetically attenuated parasites (GAPs) have been created[56-58]. Since GAPs could be made to arrest at virtually any timepoint in the developmental cycle, they could be more immunogenic than RAS without sacrificing safety[59].

The first *Pf* GAP lacked the and *p36* and *p52* genes, which code for a putative GPI-anchored protein and a putative secreted protein, respectively. Despite displaying normal infectivity, this double knock-out (*Pf* GAP2KO) showed an attenuated phenotype *in vitro* and *in vivo*. Parasites halted development in hepatocytes after 4-6 days and were absent in the livers of humanized mice after four days, as measured by microscopy and quantitative polymerase chain reaction (qPCR)[60]. However, when the *Pf* GAP2KO was tested in humans, it was incompletely attenuated and caused a breakthrough infection in one out of six exposed participants[61].

To reduce the likelihood of breakthrough, a new GAP, lacking sporozoite asparagine-rich protein 1 (*sap1*) in addition to *p52* and *p36,* was generated and termed *Pf* GAP3KO due to the triple gene deletion. Gametogenesis and sporogenesis, as well as gliding ability, hepatocyte transversal and infection *in vitro* were comparable to WT[62].

In a trial assessing *Pf* GAP3KO's efficacy, healthy participants received 200 mosquito bites per exposure: ten of them were immunized five times and six of them three times. Upon CHMI four weeks later, protection rate was 50% in both groups[63].

We and others generated one of the first GAPs, lacking the genes *b9* and *slarp (PfΔb9Δslarp*) and henceforth referred to as GA1[57]. B9 is a member of the 6-cysteine domain protein family which is fundamental for liver invasion[64], while sporozoite and liver stage asparagine-rich protein (SLARP) is an asparagine-rich protein that regulates gene expression in the liver stage[65]. GA1 parasites infected primary human hepatocytes *in vitro* but then became undetectable on days 2-7 post-infection. Similarly, when the presence of the parasite was assessed by qPCR in the livers of humanized mice, it was detected at 24 hours after exposure, but was absent four days later[57]. In the subsequent clinical trial, two different doses were tested: 4.5×10^5 or 9×10^5 cryopreserved sporozoites were administered three times at 56-day-intervals to 13 individuals each. After CHMI three weeks later, three participants were protected in total, one in the low dose and two in the high dose group. Hence, albeit safe and well-tolerated, the efficacy of GA1 was lower than desired[34].

The discovery of late-liver stage genes has opened the way to the creation of late-arresting GAPs[66]. The idea is that, similar to CPS, the longer developmental timeframe in combination with the increased antigenic repertoire and load, could elicit a stronger immune response and therefore lead to improved protection[59] (**Fig. 2**). If these GAPs were confirmed to have higher efficacy than their predecessors, they could be a new generation of malaria vaccine candidates.

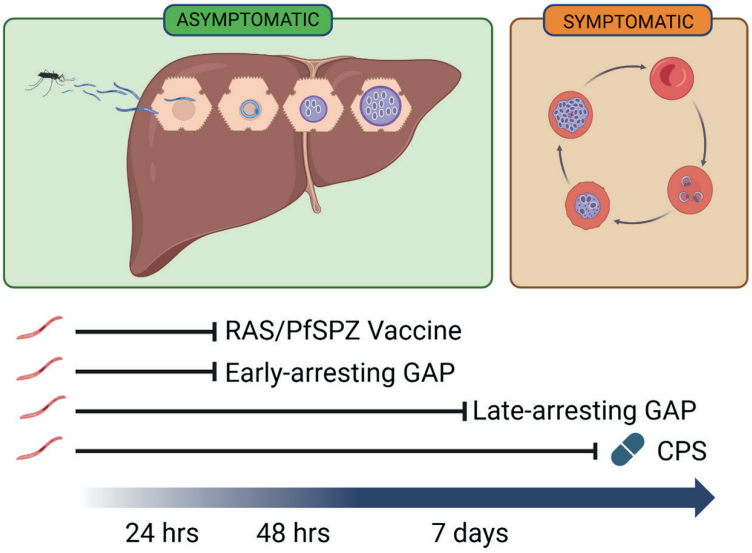


Figure 2. Development of GA2
Comparison between the development of radiation attenuated sporozoites (RAS/PfSPZ Vaccine), genetically attenuated parasites (GAPs) that arrest early or late in the liver stage and sporozoites administered in combination with chemoprophylaxis (CPS).

Clinical trials for vaccine development

The importance of human volunteers

The impact of clinical trials for scientific advancement is undeniable. Clinical research is essential to the approval of new drugs and vaccines and would not be possible without medical volunteers. However, clinical research involving humans has been a long matter of discussion. The horrors of the Second World War, characterized by coerced experimentation on prisoners, resulted in the Nürnberg Code (1947), which was later expanded and revised in the Declaration of Helsinki (1964)[67,68]. Prompted by the Tuskegee Syphilis Study[69], the Belmont Report (1978) re-defined the guidelines for safe research in human subjects[70].

The basic principles underlaying all of these documents is that participation in research should always be voluntary and that potential side-effects for the individual need to be

carefully weighed against the benefits for society. When in doubt, the welfare of the individual should always outweigh other considerations[67,68,70].

Despite multiple attempts at regulation, research involving human subjects remains problematic, especially if it is conducted on healthy individuals. In contrast to phase 2 and 3 studies, phase 1 participants experience no personal advantage and may even suffer health-related complications as a consequence of their participation. In order to make participation more attractive, medical volunteers are financially rewarded. The principal concern then, is that economically vulnerable individuals might feel pressured to participate because they receive a financial compensation[71,72]. How then can we make sure that the principle of self-determination is fulfilled in these cases?

A number of studies have tried to dispel fears by showing that clinical trial participants have mixed motivations, including, but not limited to financial compensation. The desire to help others and to aid scientific advancement are also important factors influencing the decision to participate in a clinical trial[73,74].

Much research has tried to unravel the motivations of medical volunteers, but much discussion still abides. In order to safeguard the ethics of clinical trials involving humans, it is important to understand the underlaying motivations of participants and to keep the discussion alive. It is the awareness of the question and not so much the answer that helps protect the ethical norm and the rights of medical volunteers.

Controlled human infection studies

Controlled human infection (CHI) studies are a form of clinical research which consist of the deliberate inoculation of healthy participants with infectious agents in a controlled setting, where the combination of early detection through good diagnostics and the existence of an effective therapy minimizes side effects and prevents long-term sequelae[75]. CHI studies are a research model that allows for early efficacy assessment of new experimental treatments and vaccines, saving both economic and time resources before proceeding to more complex stage 3 trials[76]. Additionally, CHI studies can provide insights into the pathophysiology of diseases in a way that other research could not[77]. Human challenges with unattenuated pathogens may be especially useful when a rapid response to new infectious threat is required, such as in the context of a pandemic[77,78].

However, despite their many advantages, CHI studies fall under intensive scrutiny as some may suggest that they are more dangerous than traditional phase 1 clinical trials with higher rates of negative side effects and exposure to diseases for which there may not be a cure (yet)[79].

Given these polarizing views on the subject and the extraordinary potential of CHI studies in understanding the pathophysiology of new emerging disease and developing effective vaccines and treatments against them, it is worth exploring whether CHI studies really differ from phase 1 clinical trials and if so, how.

Thesis outline

The scientific part of this thesis is divided into two parts: the first part consists of articles in which I was the main investigator, whereas the second one contains collaborative projects. I chose these particular collaborative projects to be included in the thesis above others because I consider them part of my formative development as a scientist and/or because I deem them important to understand the contents of this thesis.

Chapter 1 describes the first administration of GA2 to humans. The novel GAP was not only safe and well-tolerated but also had a protective efficacy of 89% against challenge with WT parasites. Moreover, we found a correlation between $CD4^+$ T cells and $\gamma\delta$ T cells and protection.

Chapter 2 puts the GA2 in the broader context of other existing GAPs, provides a comprehensive overview of the field and includes recommendations for the future.

Chapter 3 focuses on the motivations and the decision-making process of SARS-CoV2 vaccine study participants. We found that this subgroup of medical volunteers was mostly motivated by the desire to help others. We propose a theoretical framework to explain how this reasoning shift came about.

Chapter 4 is an opinion piece concerning the (perceived) difference in risk between phase 1 clinical trials and CHI studies. It provides general guidelines to attempt to mitigate risks when carrying out the latter.

Chapter 5 and **Chapter 6** are collaborative projects. **Chapter 5** describes the creation of GA2 as a new GAP and is the preclinical research that directly preceded and made the testing of GA2 in healthy human participants possible. **Chapter 6** contains the results of the research that followed the first testing of GA2 as described in Chapter 1. The safety of the parasite was confirmed once more and it was shown that a single dose of 50-GA2 infected mosquito bites is sufficient to achieve 90% protection against challenge with WT parasites.

Finally **Chapter 7** contains a brief summary and extensive discussion on the subjects of the preceding chapters.

References

1 Ashley, E. A., Pyae Phyo, A. & Woodrow, C. J. Malaria. *Lancet* **391**, 1608–1621 (2018). https://doi.org/10.1016/S0140-6736(18)30324-6

2 Howick, V. M. *et al.* The Malaria Cell Atlas: Single parasite transcriptomes across the complete Plasmodium life cycle. *Science* **365** (2019). https://doi.org/10.1126/science.aaw2619

3 Liu, Z., Miao, J. & Cui, L. Gametocytogenesis in malaria parasite: commitment, development and regulation. *Future Microbiol* **6**, 1351–1369 (2011). https://doi.org/10.2217/fmb.11.108

4 Venugopal, K., Hentzschel, F., Valkiunas, G. & Marti, M. Plasmodium asexual growth and sexual development in the haematopoietic niche of the host. *Nat Rev Microbiol* **18**, 177–189 (2020). https://doi.org/10.1038/s41579-019-0306-2

5 Cockburn, I. A. & Seder, R. A. Malaria prevention: from immunological concepts to effective vaccines and protective antibodies. *Nat Immunol* **19**, 1199–1211 (2018). https://doi.org/10.1038/s41590-018-0228-6

6 van der Heyde, H. C., Nolan, J., Combes, V., Gramaglia, I. & Grau, G. E. A unified hypothesis for the genesis of cerebral malaria: sequestration, inflammation and hemostasis leading to microcirculatory dysfunction. *Trends Parasitol* **22**, 503–508 (2006). https://doi.org/10.1016/j.pt.2006.09.002

7 WHO. World malaria report 2024: addressing inequity in the global malaria response., (2024).

8 Gonzalez-Sanz, M., Berzosa, P. & Norman, F. F. Updates on Malaria Epidemiology and Prevention Strategies. *Curr Infect Dis Rep*, 1–9 (2023). https://doi.org/10.1007/s11908-023-00805-9

9 Dondorp, A. M. *et al.* Artemisinin resistance in Plasmodium falciparum malaria. *N Engl J Med* **361**, 455–467 (2009). https://doi.org/10.1056/NEJMoa0808859

10 Xie, S. C., Ralph, S. A. & Tilley, L. K13, the Cytostome, and Artemisinin Resistance. *Trends Parasitol* **36**, 533–544 (2020). https://doi.org/10.1016/j.pt.2020.03.006

11 Rosenthal, P. J. *et al.* The emergence of artemisinin partial resistance in Africa: how do we respond? *Lancet Infect Dis* (2024). https://doi.org/10.1016/S1473-3099(24)00141-5

12 Ogunah, J. A., Lalah, J. O. & Schramm, K. W. Malaria vector control strategies. What is appropriate towards sustainable global eradication? *Sustain Chem Pharm* **18** (2020). https://doi.org/10.1016/j.scp.2020.100339

13 Pryce, J., Medley, N. & Choi, L. Indoor residual spraying for preventing malaria in communities using insecticide-treated nets. *Cochrane Database Syst Rev* **1**, CD012688 (2022). https://doi.org/10.1002/14651858.CD012688.pub3

14 Colon-Gonzalez, F. J. *et al.* Projecting the risk of mosquito-borne diseases in a warmer and more populated world: a multi-model, multi-scenario intercomparison modelling study. *Lancet Planet Health* **5**, e404–e414 (2021). https://doi.org/10.1016/S2542-5196(21)00132-7

15 Liu, N. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annu Rev Entomol* **60**, 537–559 (2015). https://doi.org/10.1146/annurev-ento-010814-020828

16 Ippolito, M. M., Moser, K. A., Kabuya, J. B., Cunningham, C. & Juliano, J. J. Antimalarial Drug Resistance and Implications for the WHO Global Technical Strategy. *Curr Epidemiol Rep* **8**, 46–62 (2021). https://doi.org/10.1007/s40471-021-00266-5

17 van der Pluijm, R. W., Tripura, R. & Hoglund, R. M. Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated Plasmodium falciparum malaria: a multicentre, open-label, randomised clinical trial (vol 395, pg 1345, 2020). *Lancet* **395**, 1344–1344 (2020). https://doi.org/10.1016/S0140-6736(20)30738-8

18 Hemingway, J. *et al.* Averting a malaria disaster: will insecticide resistance derail malaria control? *Lancet* **387**, 1785–1788 (2016). https://doi.org/10.1016/S0140-6736(15)00417-1

19 WHO. Global technical strategy for malaria 2016-2030, 2021 update. (2021).

20 Hussein, I. H. *et al.* Vaccines Through Centuries: Major Cornerstones of Global Health. *Front Public Health* **3** (2015). https://doi.org/10.3389/fpubh.2015.00269

21 Greenwood, B. The contribution of vaccination to global health: past, present and future. *Philos Trans R Soc Lond B Biol Sci* **369**, 20130433 (2014). https://doi.org/10.1098/rstb.2013.0433

22 Doolan, D. L., Dobano, C. & Baird, J. K. Acquired immunity to malaria. *Clin Microbiol Rev* **22**, 13–36, Table of Contents (2009). https://doi.org/10.1128/CMR.00025-08

23 Gonzales, S. J. *et al.* Naturally Acquired Humoral Immunity Against Plasmodium falciparum Malaria. *Front Immunol* **11**, 594653 (2020). https://doi.org/10.3389/fimmu.2020.594653

24 Langhorne, J., Ndungu, F. M., Sponaas, A. M. & Marsh, K. Immunity to malaria: more questions than answers. *Nature Immunology* **9**, 725–732 (2008). https://doi.org/10.1038/ni.f.205

25 Ryg-Cornejo, V. *et al.* Severe Malaria Infections Impair Germinal Center Responses by Inhibiting T Follicular Helper Cell Differentiation. *Cell Rep* **14**, 68–81 (2016). https://doi.org/10.1016/j.celrep.2015.12.006

26 Duffy, P. E. & Gorres, J. P. Malaria vaccines since 2000: progress, priorities, products. *Npj Vaccines* **5** (2020). https://doi.org/10.1038/s41541-020-0196-3

27 Jameson, S. C. & Masopust, D. Understanding Subset Diversity in T Cell Memory. *Immunity* **48**, 214–226 (2018). https://doi.org/10.1016/j.immuni.2018.02.010

28 Lefebvre, M. N. & Harty, J. T. You Shall Not Pass: Memory CD8 T Cells in Liver-Stage Malaria. *Trends Parasitol* **36**, 147–157 (2020). https://doi.org/10.1016/j.pt.2019.11.004

29 Kurup, S. P., Butler, N. S. & Harty, J. T. T cell-mediated immunity to malaria. *Nat Rev Immunol* **19**, 457–471 (2019). https://doi.org/10.1038/s41577-019-0158-z

30 Laidlaw, B. J., Craft, J. E. & Kaech, S. M. The multifaceted role of CD4(+) T cells in CD8(+) T cell memory. *Nat Rev Immunol* **16**, 102–111 (2016). https://doi.org/10.1038/nri.2015.10

31 Perez-Mazliah, D. & Langhorne, J. CD4T-cell subsets in malaria: TH1/TH2 revisited. *Front Immunol* **5**, 1–8 (2015). https://doi.org/ARTN 67110.3389/fimmu.2014.00671

32 Cockburn, I. A. & Seder, R. A. Malaria prevention: from immunological concepts to effective vaccines and protective antibodies. *Nature Immunology* **19**, 1199–1211 (2018). https://doi.org/10.1038/s41590-018-0228-6

33 Rts, S. C. T. P. Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites. *PLoS Med* **11**, e1001685 (2014). https://doi.org/10.1371/journal.pmed.1001685

34 Roestenberg, M. *et al.* A double-blind, placebo-controlled phase 1/2a trial of the genetically attenuated malaria vaccine PfSPZ-GA1. *Sci Transl Med* **12**, 1–9 (2020). https://doi.org/10.1126/scitranslmed.aaz5629

35 Ishizuka, A. S. *et al.* Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. *Nat Med* **22**, 614–623 (2016). https://doi.org/10.1038/nm.4110

36 Epstein, J. E. *et al.* Protection against Plasmodium falciparum malaria by PfSPZ Vaccine. *JCI Insight* **2**, 1–14 (2017). https://doi.org/10.1172/jci.insight.89154

37 Epstein, J. E. *et al.* Live Attenuated Malaria Vaccine Designed to Protect Through Hepatic CD8(+) T Cell Immunity. *Science* **334**, 475–480 (2011). https://doi.org/10.1126/science.1211548

38 Wang, L. T. *et al.* A Potent Anti-Malarial Human Monoclonal Antibody Targets Circumsporozoite Protein Minor Repeats and Neutralizes Sporozoites in the Liver. *Immunity* **53**, 733–+ (2020). https://doi.org/10.1016/j.immuni.2020.08.014

39 Wu, R. L. *et al.* Low-Dose Subcutaneous or Intravenous Monoclonal Antibody to Prevent Malaria. *N Engl J Med* **387**, 397–407 (2022). https://doi.org/10.1056/NEJMoa2203067

40 Roestenberg, M. *et al.* The frontline of controlled human malaria infections: A report from the controlled human infection models Workshop in Leiden University Medical Centre 5 May 2016. *Vaccine* **35**, 7065–7069 (2017). https://doi.org/10.1016/j.vaccine.2017.10.093

41 Polhemus, M. E. *et al.* Evaluation of RTS,S/AS02A and RTS,S/AS01B in adults in a high malaria transmission area. *PLoS One* **4**, e6465 (2009). https://doi.org/10.1371/journal.pone.0006465

42 Kester, K. E. *et al.* Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. *J Infect Dis* **200**, 337–346 (2009). https://doi.org/10.1086/600120

43 Tinto, H. *et al.* Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* **386**, 31–45 (2015). https://doi.org/10.1016/S0140-6736(15)60721-8

44 Copeland, N. K. *et al.* Efficacy of RTS,S/AS01E Only Seen in Baseline Parasitemic and Not Baseline Aparasitemic Plasmodium falciparum-Exposed, Drug-Treated Kenyan Adults. *J Infect Dis* **232**, e372–e382 (2025). https://doi.org/10.1093/infdis/jiaf274

45 Mehreen S Datoo, D. *et al.* Safety and efficacy of malaria vaccine candidate R21/Matrix-M in African children: a multicentre, double-blind, randomised, phase 3 trial. *The Lancet* **403**, 533–544 (2024).

46 Birkett, A., Miller, R. S. & Soisson, L. A. The Importance of Exercising Caution When Comparing Results from Malaria Vaccines Administered on the EPI Schedule and on a Seasonal Schedule. *Am J Trop Med Hyg* **107**, 1356–1356 (2022). https://doi.org/10.4269/ajtmh.22-0544a

47 Solana, J. C., Moreno, J., Iborra, S., Soto, M. & Requena, J. M. Live attenuated vaccines, a favorable strategy to provide long-term immunity against protozoan diseases. *Trends Parasitol* **38**, 316–334 (2022). https://doi.org/10.1016/j.pt.2021.11.004

48 Hoffman, S. L. *et al.* Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites. *J Infect Dis* **185**, 1155–1164 (2002). https://doi.org/10.1086/339409

49 Nussenzweig, R. S., Vanderberg, J., Most, H. & Orton, C. Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei. *Nature* **216**, 160–162 (1967). https://doi.org/10.1038/216160a0

50 Clyde, D. F., Most, H., McCarthy, V. C. & Vanderberg, J. P. Immunization of man against sporozite-induced falciparum malaria. *Am J Med Sci* **266**, 169–177 (1973). https://doi.org/10.1097/00000441-197309000-00002

51 Clyde, D. F., Mccarthy, V. C., Miller, R. M. & Hornick, R. B. Specificity of Protection of Man Immunized against Sporozoite-Induced Falciparum Malaria. *American Journal of the Medical Sciences* **266**, 398–403 (1973). https://doi.org/Doi 10.1097/00000441-197312000-00001

52 Clyde, D. F., Most, H., Mccarthy, V. C. & Vanderberg, J. P. Immunization of Man against Sporozite-Induced Falciparum Malaria. *American Journal of the Medical Sciences* **266**, 169–177 (1973). https://doi.org/Doi 10.1097/00000441-197309000-00002

53 Jongo, S. A. *et al.* Multi-Dose Priming Regimens of PfSPZ Vaccine: Safety and

Efficacy against Controlled Human Malaria Infection in Equatoguinean Adults. *Am J Trop Med Hyg* **106**, 1215–1226 (2022). https://doi.org/10.4269/ajtmh.21-0942

54 Jongo, S. A. *et al.* Increase of Dose Associated With Decrease in Protection Against Controlled Human Malaria Infection by PfSPZ Vaccine in Tanzanian Adults. *Clin Infect Dis* **71**, 2849–2857 (2020). https://doi.org/10.1093/cid/ciz1152

55 Roestenberg, M. *et al.* Protection against a Malaria Challenge by Sporozoite Inoculation. *New Engl J Med* **361**, 468–477 (2009). https://doi.org/DOI 10.1056/NEJMoa0805832

56 Annoura, T. *et al.* Two Plasmodium 6-Cys family-related proteins have distinct and critical roles in liver-stage development. *Faseb J* **28**, 2158–2170 (2014). https://doi.org/10.1096/fj.13-241570

57 van Schaijk, B. C. *et al.* A genetically attenuated malaria vaccine candidate based on P. falciparum b9/slarp gene-deficient sporozoites. *Elife* **3**, 1–18 (2014). https://doi.org/10.7554/eLife.03582

58 Labaied, M. *et al.* Plasmodium yoelii sporozoites with simultaneous deletion of P52 and P36 are completely attenuated and confer sterile immunity against infection. *Infect Immun* **75**, 3758–3768 (2007). https://doi.org/10.1128/IAI.00225-07

59 Nunes-Cabaco, H., Moita, D. & Prudencio, M. Five decades of clinical assessment of whole-sporozoite malaria vaccines. *Front Immunol* **13**, 1–18 (2022). https://doi.org/10.3389/fimmu.2022.977472

60 VanBuskirk, K. M. *et al.* Preerythrocytic, live-attenuated Plasmodium falciparum vaccine candidates by design. *Proc Natl Acad Sci U S A* **106**, 13004–13009 (2009). https://doi.org/10.1073/pnas.0906387106

61 Spring, M. *et al.* First-in-human evaluation of genetically attenuated Plasmodium falciparum sporozoites administered by bite of Anopheles mosquitoes to adult volunteers. *Vaccine* **31**, 4975–4983 (2013). https://doi.org/10.1016/j.vaccine.2013.08.007

62 Kublin, J. G. *et al.* Complete attenuation of genetically engineered Plasmodium falciparum sporozoites in human subjects. *Sci Transl Med* **9** (2017). https://doi.org/10.1126/scitranslmed.aad9099

63 Murphy, S. C. *et al.* A genetically engineered Plasmodium falciparum parasite vaccine provides protection from controlled human malaria infection. *Sci Transl Med* **14** (2022). https://doi.org/ARTN eabn970910.1126/scitranslmed.abn9709

64 Fernandes, P. *et al.* sporozoites require the protein B9 to invade hepatocytes. *Iscience* **26** (2023). https://doi.org/10.1016/j.isci.2023.106056

65 Silvie, O., Goetz, K. & Matuschewski, K. A sporozoite asparagine-rich protein controls initiation of Plasmodium liver stage development. *Plos Pathogens* **4** (2008). https://doi.org/10.1371/journal.ppat.1000086

66 Khan, S. M., Janse, C. J., Kappe, S. H. I. & Mikolajczak, S. A. Genetic engineering of attenuated malaria parasites for vaccination. *Curr Opin Biotech* **23**, 908–916 (2012). https://doi.org/10.1016/j.copbio.2012.04.003

67 World Medical Association. Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (1964).

68 Nuremberg Code. (1947).

69 Brandt, A. M. Racism and research: the case of the Tuskegee Syphilis Study. *Hastings Cent Rep* **8**, 21–29 (1978).

70 Department of Health, E., and Welfare. The Belmont Report. (1979).

71 Stunkel, L. & Grady, C. More than the money: A review of the literature examining healthy volunteer motivations. *Contemp Clin Trials* **32**, 342–352 (2011). https://doi.org/10.1016/j.cct.2010.12.003

72 Grady, C., Bedarida, G., Sinaii, N., Gregorio, M. A. & Emanuel, E. J. Motivations, enrollment decisions, and socio-demographic characteristics of healthy volunteers in phase 1 research. *Clin Trials* **14**, 526–536 (2017). https://doi.org/10.1177/1740774517722130

73 Hoogerwerf, M. A., de Vries, M. & Roestenberg, M. Money-oriented risk-takers or deliberate decision-makers: a cross-sectional survey study of participants in controlled human infection trials. *BMJ Open* **10**, 1–8 (2020). https://doi.org/10.1136/bmjopen-2019-033796

74 Marsh, A. A. *et al.* Characterizing altruistic motivation in potential volunteers for SARS-CoV-2 challenge trials. *PLoS One* **17**, e0275823 (2022). https://doi.org/10.1371/journal.pone.0275823

75 Roestenberg, M., Hoogerwerf, M. A., Ferreira, D. M., Mordmuller, B. & Yazdanbakhsh, M. Experimental infection of human volunteers. *Lancet Infect Dis* **18**, e312–e322 (2018). https://doi.org/10.1016/S1473-3099(18)30177-4

76 ZonMw. Controlled Human Infection Studies in the Netherlands - Roadmap. (2023).

77 WHO. WHO guidance on the ethical conduct of controlled human infection studies. (Geneva, 2021).

78 *European partnership for pandemic preparedness* <https://beready4pandemics.eu/> (

79 Jamrozik, E. & Selgelid, M. J. COVID-19 human challenge studies: ethical issues. *Lancet Infect Dis* **20**, e198–e203 (2020). https://doi.org/10.1016/S1473-3099(20)30438-2