Universiteit Leiden
The Netherlands

Safety and efficacy after immunization with Plasmodium falciparum sporozoites in the controlled human malaria infection model

Schats, R.

Citation
Schats, R. (2019, November 12). *Safety and efficacy after immunization with Plasmodium falciparum sporozoites in the controlled human malaria infection model*. Retrieved from https://hdl.handle.net/1887/80326

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/80326

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/80326 holds various files of this Leiden University dissertation.

Author: Schats, R.
Title: Safety and efficacy after immunization with Plasmodium falciparum sporozoites in the controlled human malaria infection model
Issue Date: 2019-11-12

Ghana. July 2004. Hospital Staff

CHAPTER 1

Introduction

1 INTRODUCTION

Despite recent successes, malaria remains a serious public health problem affecting approximately 40% of the world's population. Children and pregnant woman are the most vulnerable groups for severe disease. In 2015 the global incidence of malaria was estimated to be around 214 million clinical cases resulting in 438,000 deaths annually [1]. Africa is the most affected continent with more than 88% of all deaths globally, and among children it is the fourth highest cause of death. Ten percent of all child deaths in sub-Saharan Africa are attributable to malaria [1].

The development of effective field-applicable vaccines against malaria has proven to be extremely difficult. Firstly, this is due to the fact that it is still unknown which *Plasmodium* antigens and host immunological pathways are involved in the acquisition of sterile protection. Secondly, *Plasmodium* has evolved under continuous immunological selective pressure which resulted in a huge genetic diversity with subsequent high levels of antigenic variation. These ever changing antigens resemble a continuously moving target for the host immune system, and to cover these antigens by vaccines remains therefore a true challenge. To eradicate malaria from the face of the earth, a multitude of anti-malaria tools will be needed of which a vaccine will be of utmost importance. In this thesis we tried to answer several questions central in the development of a whole sporozoite malaria vaccine.

Biology of the malaria parasite

The malaria parasite belongs to the taxum Apicomplexa, a large phylum of parasitic protists. Apicomplexan parasites are eukaryotic unicellular endoparasites and many of them are important pathogens for invertebrates and vertebrates, including humans [2]. In all hosts malaria is caused by *Plasmodium* and in humans, five species of *Plasmodium* exist: *falciparum*, *vivax*, *ovale*, *malariae* and *knowlesi*.

After inoculation in the skin by an *Anopheles* mosquito, the parasites travel within 10-15 minutes [3] via the bloodstream or lymphatic system to the liver. Upon arrival in the liver, sporozoites invade and transverse several liver cells before each parasitizing a single liver cell to proliferate and differentiate. This stage in the life-cycle of the parasite is called the pre-erythrocytic stage. Within the liver cells, parasites reside clinically silent inside parasitophorous vacuoles (PV) for 5-6 days while transforming from sporozoites via schizonts into merosomes. Directly after release from liver cells and entering the blood-

stream, these merosomes release thousands of merozoites that rapidly enter (less than 30 seconds) erythrocytes [4]. Each merozoite transforms and divides via the trophozoite and schizont stage into merozoites by clonal multiplication. These merozoites are released by bursting of the erythrocyte and this cycle takes one to three days depending on the *Plasmodium* species. Simultaneously with the release of merozoites in the bloodstream, symptoms of malaria start to occur in the infected individual. Symptoms of uncomplicated malaria include flu-like symptoms like headache, fever and myalgia. Newly released merozoites again infect erythrocytes, perpetuating the cycle of infections and billions of parasites are formed. When left untreated, disease can worsen to complicated malaria and can include coma, shock, severe anaemia and can lead to death. High mortality rates can occur, especially in *Plasmodium falciparum* infections, in young infants and immune-naïve adults like travellers, pregnant women and people living in endemic areas with unstable transmission. Gametocytes are the sexual forms and are formed after several cycles of erythrocytic asexual multiplication. These gametocytes can be taken up by mosquitoes through bites allowing transmission of the disease. Only few circulating gametocytes are necessary for transmission and even if the gametocyte density in the bloodstream is as low as 1 parasite per µL, transmission remains fully possible [5]. Currently only few drugs are able to effectively kill gametocytes [6] and developing a vaccine against these sexual stages is important to further optimize vaccine effectivity of malaria control programs.

Combat against Malaria

The incidence of the individual species varies, but *P. falciparum* and *P. vivax* are primarily responsible for most of the morbidity and mortality, and most deaths are attributable to *P. falciparum* [1]. In the 1990s, the incidence of malaria increased dramatically, which was largely due to a rise in chloroquine-resistant parasites after decades of massive (mono-therapy) drug use across Asia and Africa. This changed after 1998 when the Director General Gro Harlem Brundtland called to "Roll Back Malaria" in his speech at the 51st World Health Assembly in Geneva [http://www.malaria.org/SPEECH.HTM].

Effective introduction and distribution of artemisinin-combination therapy (ACT), long-lasting Insecticide Treated Nets (ITN), Indoor Residual Spraying (IRS) and other tools to prevent malaria infection have resulted in a 30% reduction in malaria cases and a 47% reduction in deaths since 2000 [1]. Despite the implementation of ACT in many affected countries, artemisinine-resistant parasites are currently rapidly spreading across South East Asia. This is mainly due

to the use of artemisinin mono-therapy [7-9] and counterfeit poor quality anti-malarials [10]. Additionally, mosquitoes are becoming increasingly resistant to insecticides such as pyrethroids making the use of ITN and IRS less effective [11].

To reduce the incidence of (drug-resistant) malaria, a sustainable implementation of several effective anti-malaria tools is needed. These tools should minimally include adequate diagnosis and treatment, use of ITN, IRS, and vaccine development [1]. Although all elements might be equally important in the fight against malaria, the development of an effective vaccine, is not only essential but probably also the most cost-effective tool to combat malaria especially when integrated in existing expanded immunization programmes (EPI) for children [12].

Malaria vaccines

Up to this day, effective vaccines against parasites do not exist in humans [13]. The combination of the highly complex biology and the high degree of stage specific variation of surface antigens of the parasite makes vaccine development extremely challenging. Despite the fact that acquisition of natural immunity to malaria is possible, it requires years of repeated infections before an individual acquires protective IgG antibody responses against blood-stage *Plasmodium* [14, 15].

These antibody responses are able to control the number of parasites in the body, can prevent clinical malaria and reduce the risk of death. However, sterile protection is usually not accomplished under these circumstances [16] and people living in endemic areas often carry low-density parasitaemia generating symptomatic clinical episodes throughout their lives. The parasite benefits from this intricate (immunological) relationship. This interaction results in a state of chronic infection in the host without (excessive) clinical symptoms or death, and thereby facilitates continuous transmission of parasites.

In 2006 the PATH Malaria Vaccine Technology Roadmap initiative set out two goals for future vaccine development: a vaccine by 2015 that is 50% effective against severe disease and death and by 2025 a vaccine that reduces clinical malaria episodes by 80% [17]. Unfortunately, the first goal has not been realised yet. Ideally, vaccines against malaria should induce sterile protection and prevent both disease in infected individuals as well as block transmission to others. This can be achieved at several stages of the parasite life cycle in the human host; vaccines could target the parasite at the skin, liver or blood stage, or a combination of these stages (**Figure 1.1**). A vaccine blocking sporozoites at the skin or liver stage would prevent disease in an individual by killing or arrest-

ing the parasite in the earliest stages of infection. At the blood stage, vaccines could target blood-stage antigens that in its turn could eliminate blood-stage parasites and prevent disease, or target sexual stages that block transmission.

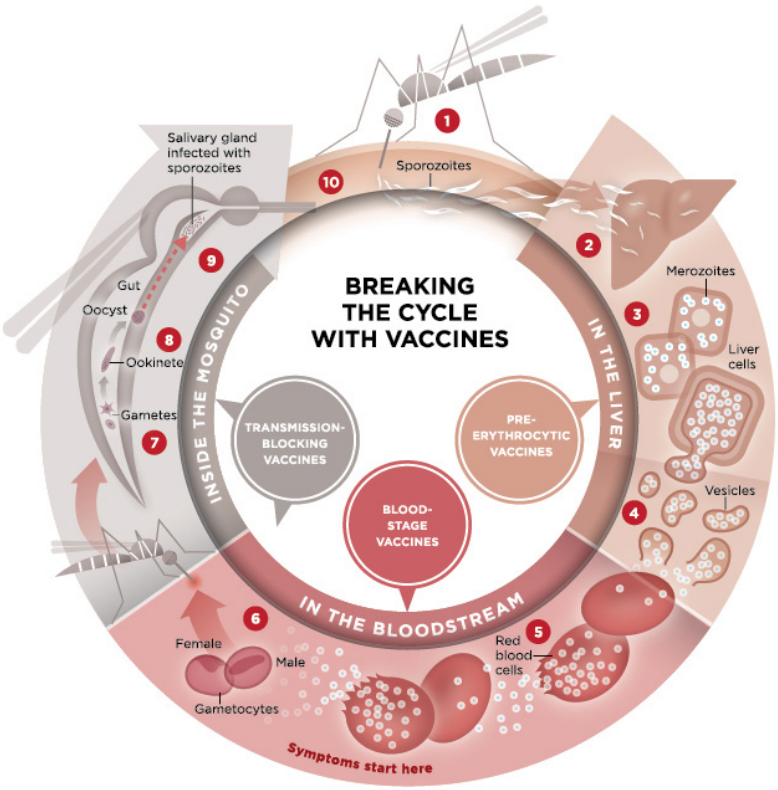

Figure 1.1 *Breaking the cycle with vaccines. Malaria Vaccine Initiative (MVI) PATH http://www.malariavaccine.org/malvac-lifecycle.php*

Although a wide range of vaccine initiatives are currently tested in clinical trials, only the RTS,S/AS01E subunit vaccine is currently being deployed in Africa [WHO Rainbow tables 2018]. RTS,S/AS01E consists of a Circumsporozoite Protein (CSP) antigen linked to the viral envelope surface protein of hepatitis B, and is administered together with the adjuvant AS01E to boost immune responses. Although RTS,S is the first licensed and distributed vaccine against malaria [18], data show a relatively low vaccine efficacy of 27%, especially under field conditions in young children [19, 20].

In addition to using subunit vaccine antigens to induce sterile protection, also whole, live, *P. falciparum* parasites can be used for vaccination. Already in the late sixties, sterile protection was established in a murine model using irradiated *P. berghei* parasites for immunisation [21]. The overall protective efficacy against a challenge with 1000 viable sporozoites was 59% between 12 and 19 days after immunization with 75.000 irradiated sporozoites.

In 1973 similar results were demonstrated with *P. falciparum* in humans [22]. However, to induce 100% protective immunity in humans, more than 1000 bites of irradiated *P. falciparum*-infected mosquitoes were needed [23]. More recently, similar results were obtained by intravenous injection of 5 times (four week interval) 1.35 x 10^5 radiation-attenuated aseptic, purified, cryopreserved sporozoites (*PfSPZ*) [24]. A challenge infection one year after immunizations conferred full homologous protection in 5 out of 5 subjects [25].

The Chemoprophylaxis Sporozoites model (CPS)

When whole sporozoites are used for vaccination, parasite development typically needs to be arrested during the parasite life cycle before symptoms or disease occur. Beside irradiation (RAS) [21], other modes of attenuation or inactivation of parasites before, during or shortly after the liver stage are possible: chemical attenuation (CAP) [26], heat [27], and genetic modification (GAP) [28].

Chemical attenuation or inactivation of blood-stage parasites can be achieved by administering blood-stage antimalarial drugs to subjects during or after inoculation with whole sporozoites. This allows the immune system to be exposed to a sufficient level and diversity of liver stage antigens for acquisition of protective immunity. This principle was first demonstrated in the murine model in 2004 using two intravenous injections of each 20.000 *P. yoellii* sporozoites under chloroquine chemoprophylaxis, and resulted in 100% protection against experimental infection [29]. Similar results were obtained in humans with the so-called Chemoprophylaxis Sporozoites model (CPS). CPS involves repeated exposure to *P. falciparum*-sporozoites infected mosquito bites under malaria chemoprophylaxis [30]. CPS-has proven to be highly effective and reproducible: three immunizations with 15 *Plasmodium*-infected mosquito bites each under chloroquine cover resulted in 100% sterile homologous protection against infection with *P. falciparum* NF54 strain [31]. Moreover, re-challenge of a subset of these subjects 48 months later showed long-lasting homologous sterile protection in 4 out of 6 subjects [32].

Controlled Human Malaria Infection model (CHMI)

Malaria vaccines candidates can be evaluated using a Controlled Human Malaria Infection model (CHMI) where small groups of malaria-naïve volunteers are immunized and subsequently challenged with a *P. falciparum* strain to assess efficacy and to evaluate reactogenicity and immunogenicity. Worldwide more than 1,300 volunteers have participated in the CHMI [33].

Besides from comparing the number of protected to unprotected individuals after challenge, vaccine efficacy in CHMI can also assessed by measuring the prepatent period in unprotected individuals. The prepatent period is the time between the challenge infection and the detection of parasites in the blood stream. Blood stream parasites can be detected in several ways, and traditionally microscopic examination of blood smears is used. A significant but incomplete elimination of the liver stage parasites will result in a prolonged prepatent period [34].

Aims of this thesis

In this thesis we evaluated efficacy, safety, and parasitological and immunological aspects of CPS using the Controlled Human Malaria Infection model.

CPS has proven to be highly effective and reproducible: three immunizations with 15 *Plasmodium*-infected mosquito bites each under chloroquine cover resulted in 100% sterile homologous protection against *P. falciparum* malaria [23]. In **Chapter 2** we determine the minimal number of infectious bites required to confer full sterile protection in a dose de-escalation immunization scheme. In CPS sterile immunity is acquired during the liver stage of the life-cycle of the parasite [35]. Although the exact mechanism how the induction of sterile protection is mediated is unknown, it is known that cytotoxic CD8+ T-cells, in association with IFNγ, IL2, TNF, granzymes and other cytotoxic mediators, play an important role in acquisition of pre-erythrocytic protection in mice [36], primates [37] and in humans [38]. However, the exact mechanism of T-cell mediated cytotoxic killing and related immunological mechanisms of protection remain to be elucidated further. In **Chapter 2** we compare cellular immune responses in protected and unprotected individuals to elucidate these T-cell mediated cytotoxic immune response associated with protection.

Chloroquine (CQ) possesses immune-modulatory properties and is able to enhance $CD8^+$ T cell responses by induction of cross-presentation [39]. Because of these properties, CQ could have boosted immune responses and may have aided in the acquisition of sterile protection in CPS. However, due to the current worldwide CQ resistance of *P. falciparum*, the use of CQ in CPS

may be limited, and the efficacy of other *P. falciparum* blood-stage chemoprophylaxis for future field vaccinations with immunizing strains resistant to CQ needs to be assessed. Therefore, we compare in **Chapter 3** the ability of CQ and mefloquine (MQ) to induce sterile protection in CPS. MQ is one of few other blood-stage anti-malarial drugs that theoretically could replace CQ in CPS. MQ, a quinine-related schizonticidal antimalarial drug, was developed during the Vietnam War in order to counteract the rapid and widespread emergence of resistance to CQ. MQ has been widely used as chemoprophylaxis in travellers and businessmen to allow travel to areas with CQ-resistant *falciparum* malaria [40] [41]. MQ has similar mode of action as CQ, but it lacks the immune modulatory properties. MQ targets blood stage malaria parasites without affecting proliferation of liver stage parasite.

Worldwide, the *P. falciparum* NF54 strain has been most often used to immunize and challenge volunteers [42]. The *P. falciparum* NF54 strain is a laboratory strain, obtained from a case of airport malaria in het Netherlands, and originates most probably from West-Africa. The NF54 strain is sensitive to chloroquine, mefloquine, atovaquone/proguanil and arthemeter/lumefantrine.

However, in malaria-endemic areas there is a large genetic and antigenic diversity between *P. falciparum* strains. It is unclear to what extent diversity in immunizing strains is required for the development of a sufficient heterologously protective malaria vaccine [43]. Previously, heterologous protection has only been reported in 4 out of 6 RAS-immunized volunteers [44], but this required large numbers of mosquito bites. Assessing heterologous protection is essential for future deployment of these vaccines in the field. In **Chapter 4**, we assess heterologous protection against a *P. falciparum* NF135 strain, originating from Cambodia [42]. A subset of volunteers who had previously participated in the dose de-escalation NF54 CPS-immunization and homologous challenge trial described in Chapter 1 were re-challenged with the NF135 strain to assess heterologous protection after more than one year.

During CHMI the presence of blood stage parasites is traditionally detected by microscopic examination of thick blood smears. A more accurate and sensitive tool is PCR. Real-time quantitative PCR (qPCR) can detect parasite DNA before being detectable by microscopic examination, and this is called the sub-microscopic period. Parasite DNA can be detected as early as 6 days after challenge. The length of the pre-patent period is associated with the level of relative protection. In addition, the use of qPCR allows for studying the kinetics of parasite multiplication by statistical modeling.

The introduction of the more sensitive qPCR instead of thick smear for the determination of the pre-patent period will also result in earlier treatment of volunteers in CHMI, with less blood-stage parasites and fewer adverse events

(AE). In **Chapter 5** we explore the dynamics of parasitaemia and adverse events during immunizations and after challenge and the consequences if qPCR was used to initiate treatment using the two clinical trials described in **Chapters 2 and 3**. In **Chapter 6** we assess the use of qPCR as a primary diagnostic test and provide directions on how to operate and to collect parasitological and immunological data in CHMIs in the future.

REFERENCES

1. WHO Malaria Report 2015.
2. Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria. Clinical microbiology reviews. 2002;15(4):564-94. PubMed PMID: 12364370; PubMed Central PMCID: PMC126857.
3. Sidjanski S, Vanderberg JP. Delayed migration of Plasmodium sporozoites from the mosquito bite site to the blood. The American journal of tropical medicine and hygiene. 1997;57(4):426-9. PubMed PMID: 9347958.
4. Gilson PR, Crabb BS. Morphology and kinetics of the three distinct phases of red blood cell invasion by *Plasmodium falciparum* merozoites. International journal for parasitology. 2009;39(1):91-6. doi: 10.1016/j.ijpara.2008.09.007. PubMed PMID: 18952091.
5. White NJ. The role of anti-malarial drugs in eliminating malaria. Malaria journal. 2008;7 Suppl 1:S8. doi: 10.1186/1475-2875-7-S1-S8. PubMed PMID: 19091042; PubMed Central PMCID: PMC2604872.
6. Dechy-Cabaret O, Benoit-Vical F. Effects of antimalarial molecules on the gametocyte stage of Plasmodium falciparum: the debate. Journal of medicinal chemistry. 2012;55(23):10328-44. doi: 10.1021/jm3005898. PubMed PMID: 23075290.
7. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. The New England journal of medicine. 2009;361(5):455-67. doi: 10.1056/NEJMoa0808859. PubMed PMID: 19641202; PubMed Central PMCID: PMC3495232.
8. WHO. Status report on artemisinin resistance: September 2014. Geneva: World Health Organization. 2014.
9. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. The New England journal of medicine. 2014;371(5):411-23. doi: 10.1056/NEJMoa1314981. PubMed PMID: 25075834; PubMed Central PMCID: PMC4143591.
10. Kelesidis T, Falagas ME. Substandard/counterfeit antimicrobial drugs. Clinical microbiology reviews. 2015;28(2):443-64. doi: 10.1128/CMR.00072-14. PubMed PMID: 25788516; PubMed Central PMCID: PMC4402958.
11. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends in parasitology. 2011;27(2):91-8. doi: 10.1016/j.pt.2010.08.004. PubMed PMID: 20843745.
12. Asante KP, Abdulla S, Agnandji S, Lyimo J, Vekemans J, Soulanoudjingar S, et al. Safety and efficacy of the RTS,S/AS01E candidate malaria vaccine given with expanded-programme-on-immunisation vaccines: 19 month follow-up of a randomised, open-label, phase 2 trial. The Lancet infectious diseases. 2011;11(10):741-9. doi: 10.1016/S1473-3099(11)70100-1. PubMed PMID: 21782519.
13. Plotkin S. History of vaccination. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(34):12283-7. doi: 10.1073/pnas.1400472111. PubMed PMID: 25136134; PubMed Central PMCID: PMCPMC4151719.

14. Schofield L, Mueller I. Clinical immunity to malaria. Curr Mol Med. 2006;6(2):205-21. PubMed PMID: 16515511.

15. Cohen S, Mc GI, Carrington S. Gamma-globulin and acquired immunity to human malaria. Nature. 1961;192:733-7. PubMed PMID: 13880318.

16. Doolan DL, Dobano C, Baird JK. Acquired immunity to malaria. Clinical microbiology reviews. 2009;22(1):13-36, Table of Contents. doi: 10.1128/CMR.00025-08. PubMed PMID: 19136431; PubMed Central PMCID: PMC2620631.

17. Path. Malaria vaccin technology roadmap. 2006.

18. Birkett AJ, Moorthy VS, Loucq C, Chitnis CE, Kaslow DC. Malaria vaccine R&D in the Decade of Vaccines: breakthroughs, challenges and opportunities. Vaccine. 2013;31 Suppl 2:B233-43. doi: 10.1016/j.vaccine.2013.02.040. PubMed PMID: 23598488.

19. Rts SCTP. 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 medicine. 2014;11(7):e1001685. doi: 10.1371/journal.pmed.1001685. PubMed PMID: 25072396; PubMed Central PMCID: PMC4114488.

20. Olotu A, Fegan G, Wambua J, Nyangweso G, Awuondo KO, Leach A, et al. Four-year efficacy of RTS,S/AS01E and its interaction with malaria exposure. The New England journal of medicine. 2013;368(12):1111-20. doi: 10.1056/NEJMoa1207564. PubMed PMID: 23514288.

21. Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei. Nature. 1967;216(5111):160-2. PubMed PMID: 6057225.

22. Clyde DF, Most H, McCarthy VC, Vanderberg JP. Immunization of man against sporozite-induced falciparum malaria. The American journal of the medical sciences. 1973;266(3):169-77. PubMed PMID: 4583408.

23. Hoffman SL, Goh LM, Luke TC, Schneider I, Le TP, Doolan DL, et al. Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. The Journal of infectious diseases. 2002;185(8):1155-64. doi: 10.1086/339409. PubMed PMID: 11930326.

24. Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science. 2013;341(6152):1359-65. doi: 10.1126/science.1241800. PubMed PMID: 23929949.

25. Ishizuka AS, Lyke KE, DeZure A, Berry AA, Richie TL, Mendoza FH, et al. Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. Nature medicine. 2016. doi: 10.1038/nm.4110. PubMed PMID: 27158907.

26. Purcell LA, Yanow SK, Lee M, Spithill TW, Rodriguez A. Chemical attenuation of Plasmodium berghei sporozoites induces sterile immunity in mice. Infection and immunity. 2008;76(3):1193-9. doi: 10.1128/IAI.01399-07. PubMed PMID: 18174336; PubMed Central PMCID: PMCPMC2258828.

27. Alger NE, Harant J. Plasmodium berghei: heat-treated sporozoite vaccination of mice. Experimental parasitology. 1976;40(2):261-8. PubMed PMID: 786707.

28. Halbroth BR, Draper SJ. Recent developments in malaria vaccinology. Adv Parasitol. 2015;88:1-49. doi: 10.1016/bs.apar.2015.03.001. PubMed PMID: 25911364.

29. Belnoue E, Costa FT, Frankenberg T, Vigario AM, Voza T, Leroy N, et al. Protective T cell immunity against malaria liver stage after vaccination with live sporozoites under chloroquine treatment. Journal of immunology. 2004;172(4):2487-95. PubMed PMID: 14764721.

30. Sauerwein RW, Bijker EM, Richie TL. Empowering malaria vaccination by drug administration. Current opinion in immunology. 2010;22(3):367-73. doi: 10.1016/j.coi.2010.04.001. PubMed PMID: 20434895.

31. Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ, et al. Protection against a malaria challenge by sporozoite inoculation. The New England journal of medicine. 2009;361(5):468-77. doi: 10.1056/NEJMoa0805832. PubMed PMID: 19641203.

32. Roestenberg M, Teirlinck AC, McCall MB, Teelen K, Makamdop KN, Wiersma J, et al. Long-term protection against malaria after experimental sporozoite inoculation: an open-label follow-up study. Lancet. 2011;377(9779):1770-6. doi: 10.1016/S0140-6736(11)60360-7. PubMed PMID: 21514658.

33. Spring M, Polhemus M, Ockenhouse C. Controlled human malaria infection. The Journal of infectious diseases. 2014;209 Suppl 2:S40-5. doi: 10.1093/infdis/jiu063. PubMed PMID: 24872394.

34. Roestenberg M, O'Hara GA, Duncan CJ, Epstein JE, Edwards NJ, Scholzen A, et al. Comparison of clinical and parasitological data from controlled human malaria infection trials. PloS one. 2012;7(6):e38434. doi: 10.1371/journal.pone.0038434. PubMed PMID: 22701640; PubMed Central PMCID: PMC3372522.

35. Bijker EM, Bastiaens GJ, Teirlinck AC, van Gemert GJ, Graumans W, van de Vegte-Bolmer M, et al. Protection against malaria after immunization by chloroquine prophylaxis and sporozoites is mediated by preerythrocytic immunity. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(19):7862-7. doi: 10.1073/pnas.1220360110. PubMed PMID: 23599283; PubMed Central PMCID: PMCPMC3651438.

36. Van Braeckel-Budimir N, Harty JT. CD8 T-cell-mediated protection against liver-stage malaria: lessons from a mouse model. Front Microbiol. 2014;5:272. doi: 10.3389/fmicb.2014.00272. PubMed PMID: 24936199; PubMed Central PMCID: PMCPMC4047659.

37. Weiss WR, Jiang CG. Protective CD8+ T lymphocytes in primates immunized with malaria sporozoites. PloS one. 2012;7(2):e31247. doi: 10.1371/journal.pone.0031247. PubMed PMID: 22355349; PubMed Central PMCID: PMC3280278.

38. Tsuji M. A retrospective evaluation of the role of T cells in the development of malaria vaccine. Experimental parasitology. 2010;126(3):421-5. doi: 10.1016/j.exppara.2009.11.009. PubMed PMID: 19944099; PubMed Central PMCID: PMC3603350.

39. Accapezzato D, Visco V, Francavilla V, Molette C, Donato T, Paroli M, et al. Chloroquine enhances human CD8+ T cell responses against soluble antigens in vivo. The Journal of experimental medicine. 2005;202(6):817-28. doi: 10.1084/jem.20051106. PubMed PMID: 16157687; PubMed Central PMCID: PMC2212941.

40. Greenwood B. The use of anti-malarial drugs to prevent malaria in the population of malaria-endemic areas. The American journal of tropical medicine and hygiene. 2004;70(1):1-7. PubMed PMID: 14971690.

41. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. Malaria. Lancet. 2014;383(9918):723-35. doi: 10.1016/S0140-6736(13)60024-0. PubMed PMID: 23953767.

42. Teirlinck AC, Roestenberg M, van de Vegte-Bolmer M, Scholzen A, Heinrichs MJ, Siebelink-Stoter R, et al. NF135.C10: a new *Plasmodium falciparum* clone for controlled human malaria infections. The Journal of infectious diseases. 2013;207(4):656-60. doi: 10.1093/infdis/jis725. PubMed PMID: 23186785; PubMed Central PMCID: PMC3549599.

43. Takala SL, Plowe CV. Genetic diversity and malaria vaccine design, testing and efficacy: preventing and overcoming 'vaccine resistant malaria'. Parasite immunology. 2009;31(9):560-73. doi: 10.1111/j.1365-3024.2009.01138.x. PubMed PMID: 19691559; PubMed Central PMCID: PMC2730200.

44. Hoffman SL, Goh LM, Luke TC, Schneider I, Le TP, Doolan DL, et al. Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. The Journal of infectious diseases. 2002;185(8):1155-64. PubMed PMID: 11930326.