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MINIREVIEW

Controlled Human Malaria Infection: Applications, Advances, and Challenges

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ABSTRACT Controlled human malaria infection (CHMI) entails deliberate infection with malaria parasites either by mosquito bite or by direct injection of sporozoites or parasitized erythrocytes. When required, the resulting blood-stage infection is curtailed by the administration of antimalarial drugs. Inducing a malaria infection via inoculation with infected blood was first used as a treatment (malariotherapy) for neurosyphilis in Europe and the United States in the early 1900s. More recently, CHMI has been applied to the fields of malaria vaccine and drug development, where it is used to evaluate products in well-controlled early-phase proof-of-concept clinical studies, thus facilitating progression of only the most promising candidates for further evaluation in areas where malaria is endemic. Controlled infections have also been used to immunize against malaria infection. Historically, CHMI studies have been restricted by the need for access to insectaries housing infected mosquitoes or suitable malaria-infected individuals. Evaluation of vaccine and drug candidates has been constrained in these studies by the availability of a limited number of *Plasmodium falciparum* isolates. Recent advances have included cryopreservation of sporozoites, the manufacture of well-characterized and genetically distinct cultured malaria cell banks for blood-stage infection, and the availability of *Plasmodium vivax*-specific reagents. These advances will help to accelerate malaria vaccine and drug development by making the reagents for CHMI more widely accessible and also enabling a more rigorous evaluation with multiple parasite strains and species. Here we discuss the different applications of CHMI, recent advances in the use of CHMI, and ongoing challenges for consideration.

KEYWORDS controlled human malaria infection, drug development, experimental malaria, *Plasmodium*, vaccine development, malaria

Controlled human malaria infection (CHMI) can be undertaken either by inoculation of sporozoites via mosquito bite or by direct injection of sporozoites or *Plasmodium*-infected blood. The inoculation of sporozoites allows both liver- and blood-stage infection to develop, while induced blood-stage infection with parasitized erythrocytes results in blood-stage infection only. Blood-stage infection is truncated by antimalarial drug treatment that is initiated according to predefined study-specific criteria. Studies utilizing induced blood-stage infection typically treat infections at a predefined blood-stage parasite density (as determined by PCR) or at the onset of microscopic patency. CHMI studies involving sporozoite-initiated infections have also relied on microscopic patency as the trigger for treatment, although more recently, quantitative PCR (qPCR) has been explored as the primary test for initiating treatment in sporozoite-initiated CHMI studies (1).

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TABLE 1 Applications of controlled human malaria infection

Application	Reference(s)
Drug evaluation	
Sporozoite challenge	12, 92–106
Blood-stage challenge	9–11, 95, 96, 98–103, 105–114[a]
Vaccine evaluation	
P. falciparum preerythrocytic vaccine candidate	
Sporozoite challenge	18, 20, 36–38, 40, 42, 79, 80, 115–151
Blood-stage challenge	115
P. falciparum blood-stage vaccine candidate	
Sporozoite challenge	14, 20 36, 42, 125, 126, 131, 145, 146, 151
Blood-stage challenge	13, 16, 19
P. vivax preerythrocytic vaccine candidate	
Sporozoite challenge	15, 17, 37, 149
Blood-stage challenge	Not applicable
Immunization strategy	
Chemoprophylaxis and sporozoites	7, 8, 49–51, 81, 152
Blood-stage infection and drug treatment	6
Parasite diagnostics	21, 22
Parasite biology	153
Factors influencing virulence	23–25, 154–156
Disease processes	26, 157–159
Human immune response	27–32, 84, 86, 160–209

[a]Studies registered on the Australian New Zealand Clinical Trials Registry but not yet published: ACTRN12617000244303 and ACTRN12614000781640.

Deliberate human malaria infection with malaria parasites was initially used as a treatment (malariotherapy), for neurosyphilis in the early 1900s (reviewed in references 2 and 3). It was also used in the 1990s in limited and contentious studies as a potential treatment for HIV infection (4). From the 1940s, its utility as a tool to evaluate candidate antimalarial drugs was recognized when it was employed to assess their efficacy in healthy, nonimmune males by inoculation of *Plasmodium*-infected blood or mosquitoes (5). Since then, it has been increasingly recognized that CHMI offers a well-controlled and safe framework to undertake *in vivo* assessment of the efficacy of malaria vaccine candidates and drugs (Table 1). Researchers are also investigating the use of CHMI to immunize against malaria infection, with protection observed following multiple cycles of infection (sporozoite or blood-stage initiated) and drug treatment (see, e.g., references 6 to 8) (Table 1). Cumulative experience with CHMI over the past decades, as well as recent advances in methodologies and reagents, has resulted in the development of well-characterized experimental systems that are becoming more widely accessible to researchers working in malaria vaccine and drug development. Below, we describe the primary applications of CHMI and recent advances and highlight some of the challenges to be considered.

USES OF CONTROLLED HUMAN MALARIA INFECTION

CHMI is a valuable tool that can be used to evaluate novel antimalarial drugs (see, e.g., references 9 to 12) (Table 1), malaria vaccine candidates (see, e.g., references 7 and 13 to 20) (Table 1), and diagnostic tools (see, e.g., references 21 and 22) (Table 1). Substudies undertaken within the framework of CHMI studies for drug or vaccine evaluation have also enabled an examination of parasite biology, e.g., factors influencing virulence/disease processes (see, e.g., references 23 to 26) (Table 1) and malaria-specific human immune responses (see, e.g., references 27 to 31) (Table 1), including identification of possible immune correlates of protection (19, 31, 32). Recently, vaccine development efforts have focused on using CHMI to induce protective immunity by

truncating malaria infection at low parasitemia with drug treatment (see, e.g., references 6 to 8) (Table 1). Following multiple cycles of infection and drug treatment, protection against malaria infection has been demonstrated (6–8). Below, we discuss the main applications of CHMI: its role in drug and vaccine evaluation and as an immunization strategy against malaria infection.

Drug evaluation. Increasing levels of antimalarial drug resistance, including resistance against artemisinin-containing drugs, emphasize the urgent need for the development of new antimalarials. Following preclinical and phase I studies, phase II clinical studies are required to identify the correct dosing regimen to enable cure. CHMI in malaria-naive individuals offers a well-controlled environment to rapidly assess the efficacy of drugs with unknown therapeutic activity and to obtain pharmacokinetic and pharmacodynamics data (9–11). An additional advantage of this approach is that, providing study participants are screened appropriately, antimalarial immunity will not affect parasite clearance rates, which could lead to an overestimation of drug efficacy (33). The use of rapid and sensitive qPCR assays for parasite quantification ensures that rescue treatment with fast-acting antimalarial drugs can be administered promptly (11). Both mosquito bite- and blood-stage-initiated infections have been used in this way to evaluate antimalarial drugs (see, e.g., references 9, 12, and 34) (Table 1). While sporozoite-initiated infection is obviously required to assess the causal prophylactic activity of drugs (12, 34), one of the major advantages of induced blood-stage malaria infection for assessing parasite clearance by blood schizonticidal drugs is the standardization and precise quantification of the number of parasites initiating the blood-stage infection in each study participant.

Vaccine evaluation. Following the demonstration of safety and immunogenicity in a phase I study, undertaking phase IIa studies in areas where malaria is not endemic using CHMI enables the generation of proof-of-concept efficacy data prior to transitioning a vaccine candidate into costly phase IIb field trials in areas of endemicity (35). The efficacy of malaria vaccine candidates has been assessed using sporozoite or induced blood-stage malaria infection prior to field testing (see, e.g., references 8, 13, 18, 36, and 37) (Table 1) and for further optimization of the immunization regimen following suboptimal efficacy of a vaccine candidate in the field (38). For the former, if good efficacy is not demonstrated in phase IIa trials, this may halt progression of the vaccine candidate. For some candidates, however, one might predict an improvement in vaccine-induced protection in areas of endemicity where boosting of the vaccine-specific immune response may occur following natural exposure, or alternatively, the vaccine may augment preexisting naturally acquired immune responses. This has not yet been observed for any malaria vaccine candidate that has progressed into trials in areas where malaria is endemic, including the licensed malaria vaccine RTS,S/AS01 (39). In CHMI studies in malaria-naive humans, the endpoints for evaluation of vaccine efficacy are life cycle stage specific. For preerythrocytic vaccine candidates, the traditional study endpoint is detection of a patent blood-stage infection by microscopy. Where blood-stage infection does develop and with the use of sensitive qPCR methods, the resulting blood-stage parasitemia data can also be analyzed to obtain information on additional parasite parameters, e.g., reduction in liver load (40, 41). For blood-stage vaccine candidates, the primary assessment is the parasite multiplication rate (PMR). This can be derived from analysis of qPCR-based parasitemia data from an adequate number of time points and is used to detect differences between vaccinees and control subjects.

Historically, experimental sporozoite-initiated infection has been more widely used to evaluate vaccines in phase IIa trials and has been used predominantly for testing preerythrocytic vaccine candidates (see, e.g., references 8, 18, and 37) (Table 1), although it has been used for a small number of studies involving blood-stage vaccine candidates (14, 42). The obvious benefit of using the sporozoite-initiated infection model is that it mimics the natural route of infection; however, as it is not possible to control the number of sporozoites being inoculated by a mosquito, the challenge dose

can be highly variable (43, 44). While the use of cryopreserved, purified sporozoites delivered by needle and syringe may result in a more reproducible inoculum, further work is required to optimize this system (45).

Induced blood-stage malaria infection has also been used for evaluation of blood-stage vaccine candidates in phase IIa studies (13, 16, 19), and although a direct comparison of both CHMI models to test blood-stage vaccine efficacy has not yet been undertaken, it offers a number of advantages in malaria-naive individuals compared with sporozoite-initiated infections (46). First, being able to precisely enumerate the number of parasites initiating the blood-stage infection allows modeling of the PMR with greater accuracy, thus providing greater power to detect partial efficacy of blood-stage vaccines (19). Second, initiating a blood-stage infection with fewer parasites in the inoculum than the theoretical number of merozoites released from an infected hepatocyte can result in a prolongation of the period when submicroscopic parasitemia can be observed and measured before drug treatment is required (46, 47). Not only does this increase the number of time points at which to collect parasitemia data and thus enable a more accurate modeling of PMR, it also increases the time over which a vaccine-induced immune response can operate (and thus prevents the premature abandonment of a partially effective vaccine that could be further optimized). Similarly to the case for the sporozoite-initiated infection model, there are a number of potential shortcomings that should be considered. Viability of the injected parasites can be determined only retrospectively, making it difficult to standardize the number of viable parasites in the inoculum. Parasite viability has been shown to vary across different studies and sites (47), and this can be influenced by storage conditions and the time between thawing of the parasites and inoculation of the volunteers (46). Additionally, by circumventing the liver, induced blood-stage challenge will not detect effects on preerythrocytic parasite stages and thus may underestimate the efficacy of vaccines containing antigens that are shared between liver and blood stages.

CHMI: an immunization strategy. The use of whole parasites as a vaccine approach is advantageous, due to the broad array of antigens presented to the immune system. A number of research groups are focused on developing whole-parasite vaccines utilizing the CHMI model, which involves either sporozoite-induced malaria infection or induced blood-stage malaria infection. Different variations of CHMI are being examined, with the regimen consisting of multiple rounds of infection and drug treatment (see, e.g., references 6 to 8) (Table 1). The protective efficacy of a strategy using blood-stage CHMI has been examined in humans (6). Multiple low doses of *Plasmodium falciparum*-parasitized red blood cells were administered intravenously to malaria-naive volunteers, with each infection truncated with Malarone (atovaquone-proguanil) prior to patency (6). While parasite-specific antibodies were not detected, robust cellular immune responses were induced and protection was observed in 3 out of 4 volunteers, although it could not be excluded that residual antimalarial drug may have contributed to this protection (48). Preerythrocytic vaccine approaches utilizing live sporozoites are more advanced than blood-stage vaccine approaches, and multiple studies have examined the protective efficacy of this approach (see, e.g., references 7 and 8) (Table 1). The chemoprophylaxis and sporozoite (CPS) approach involves administering multiple mosquito-bite induced infections under chemoprophylaxis. Induction of long-lived sterile protection against homologous challenge has been demonstrated (7, 49). Drugs targeting blood stages are used in this approach to enable full liver-stage development. Although low levels of blood-stage parasitemia are observed following each infection, the protection is dependent on immune responses against the preerythrocytic stage (50). Only chloroquine and mefloquine have been utilized in humans in this model so far (51). To further advance this immunization strategy, inoculation of sporozoites by needle and syringe (discussed below) and a regimen that enables drug treatment to be administered concurrently with the parasite inoculum (sporozoite or blood stage) are critical to being able to successfully deploy this in areas where malaria is endemic. Recently, direct venous inoculation of aseptic, purified,

nonirradiated *P. falciparum* sporozoites under chloroquine cover (PfSPZ-CVac) was shown to induce sterile protective immunity against homologous challenge (8). Further approaches for vaccination include the administration of genetically attenuated sporozoites that arrest in the liver and do not progress to a blood-stage infection (52) or of blood-stage parasites which have reduced ability to replicate in the blood (53). An immunization regimen using these genetically attenuated parasites may not need administration of antimalarial drugs.

RECENT ADVANCES IN CONTROLLED HUMAN MALARIA INFECTION

Development of cryopreserved, purified sporozoites for CHMI. The traditional CHMI model involved administering bites of *Plasmodium*-infected insectary-raised mosquitoes to study participants and was standardized over decades (54), with the bites of three aseptically reared or five laboratory-reared mosquitoes consistently infecting malaria-naive individuals (55). Mosquito bite-initiated CHMI requires insectary access, entomological expertise, secure transportation of infected mosquitoes to the clinical trial site, and precise timing of mosquito rearing and infection in relation to the vaccination and challenge regimen (55). The number of sporozoites injected into each participant in mosquito bite-initiated CHMI is highly variable, and it has been shown that the number of sporozoites counted in each salivary gland/number of mosquito bites is a poor predictor of the number of sporozoites actually injected (44). A methodology has been developed by Sanaria Inc. to produce aseptic, purified, cryopreserved *P. falciparum* sporozoites that are manufactured in compliance with regulatory standards and are infective *in vivo* (56). These sporozoites are injected with a needle and syringe, and different routes of inoculation have been examined and optimized (45, 56–58). Although this artificial method of administration is clearly different from a mosquito bite and bypasses the "skin-stage," it enables a consistent sporozoite inoculum for this CHMI model to be utilized in numerous research centers around the world both for challenge and potentially for the preerythrocytic vaccine approach described above. Being able to standardize and define the number of sporozoites injected is advantageous in terms of sporozoite dose estimation for vaccine studies and enabling direct comparisons of CHMI sporozoite challenge studies between and within different clinical sites (59).

Access to *P. falciparum* material for CHMI. An important constraint to the ability to conduct CHMI is access to well-characterized malaria parasites with a known drug sensitivity profile to ensure that the most appropriate antimalarial drug treatment can be initiated when required. These parasites must also meet relevant region-specific regulatory standards so that they are suitable for administration to humans in clinical studies.

Historically, CHMI via mosquito bite or injection of sporozoites has been restricted to institutions with the capacity to rear and maintain *Plasmodium*-infected mosquitoes (36, 60–63). As outlined above, the manufacture of aseptic, purified, cryopreserved *P. falciparum* sporozoites enables this model to now be employed in numerous centers around the world (see, e.g., references 57, 59, and 64).

Blood-stage CHMI was originally developed in Australia at the Queensland Institute of Medical Research, using cryopreserved stocks of erythrocytes from two parasitemic donors who were deliberately infected with *P. falciparum* 3D7 via mosquito bite (65). This material has now been administered intravenously to >300 volunteers in numerous studies with diverse endpoints. Until recently, the use of material suitable for induced blood-stage malaria infection relied entirely upon obtaining ethical approval to collect, cryopreserve, and store a large volume of blood from suitable malaria-infected donors (either deliberately infected individuals or malaria-infected returned travelers). Following rigorous testing and ethical approval, these *ex vivo* banks could be used in clinical trials. They are, however, a finite resource, and if the donor is not of the "universal" blood group O Rh D-negative blood type, then this limits potential recipients to those with a compatible blood type. Recently, we developed an alternative approach to generating suitable blood-

stage parasites for CHMI studies (66). It involves culturing large volumes of defined *P. falciparum* isolates in blood group O Rh D-negative blood followed by cryopreservation, characterization, and rigorous testing to ensure suitability for use in clinical studies. Parasites from the different cultured *P. falciparum* blood-stage cell banks have been used to successfully infect malaria-naive human volunteers (23). The availability of CHMI reagents to multiple research centers introduces new challenges, and these are discussed further below.

***Plasmodium vivax* and CHMI.** While this review has focused largely on the use of *P. falciparum* CHMI, the recent development of *P. vivax*-specific material will accelerate the progress of vaccines and drugs specifically targeting this parasite (67, 68).

For sporozoite-initiated CHMI, published studies have utilized *P. vivax* sporozoites that were generated by feeding gametocyte-infected blood from residents of areas in Colombia and Thailand where malaria is endemic to laboratory-reared mosquitoes (15, 17, 68–70). Due to the research facilities and capacity established in Colombia, the infected mosquitoes were used in studies at the same site, whereas the mosquitoes infected in Thailand were transported in secure containers to the United States and maintained in the insectary at WRAIR until required for challenge. These *P. vivax*-infected mosquitoes were used to successfully infect malaria-naive and semi-immune individuals (68–70) and in CHMI trials evaluating *P. vivax* preerythrocytic vaccine candidates (15, 17). Due to inherent issues with long-term *in vitro* culture of *P. vivax*, there is a requirement for fresh gametocytes from infected patients to infect mosquitoes. Currently, gametocyte infection of mosquitoes is undertaken in an area where malaria is endemic, although the mosquitoes can subsequently be shipped to other centers for the challenge component of a vaccine/drug evaluation study as described above (15). The use of a different *P. vivax* isolate for each study will be reflected in parasite parameters, e.g., differential drug sensitivities, parasite multiplication rates, and prepatent periods, and this will limit comparisons between different studies (71). Until cryopreserved sporozoites are developed for *P. vivax*, this will be an ongoing limitation. A further complication of *P. vivax* sporozoite-initiated CHMI studies is the possibility of hypnozoite formation and infection relapse. Primaquine should be administered at the conclusion of the study to clear any latent liver stages. However, participants in these studies must be assessed prior to enrollment for possible exclusion based on glucose-6-phosphate dehydrogenase (G6PD) deficiency (to avoid primaquine-induced hemolysis) and for CYPD26 polymorphisms which may affect the conversion of primaquine to its active metabolite (72, 73).

There have been four *P. vivax* blood-stage CHMI studies to date, two of which have been published (67, 71, 74). These studies were undertaken using cryopreserved blood from returned travelers (67, 71), and due to the current unavailability of a long-term *in vitro P. vivax* culture, it is conceivable that *ex vivo* banks from returned travelers or deliberately infected individuals will be the only source of material for *P. vivax* blood-stage CHMI for the foreseeable future.

CHALLENGES FOR CONTROLLED HUMAN MALARIA INFECTION STUDIES

Availability of diverse parasite strains to evaluate heterologous protection. Ultimately, a malaria vaccine must induce significant strain-transcending protective efficacy. This has proven to be a challenging proposition both in the field and in CHMI studies. When evaluated in phase III trials, the licensed malaria vaccine RTS,S/AS01 demonstrated only partial protection in the field (75). Protective efficacy was shown to be greater against *P. falciparum* infections where the parasite circumsporozoite protein genotype matched that of the vaccine strain, due to the allele-specific nature of the vaccine-induced protective immune response (76). The use of defined, genetically distinct *P. falciparum* strains in CHMI studies can therefore be seen as advantageous, as they can be used to evaluate the protective efficacy of malaria vaccine candidates against a range of diverse parasite strains prior to deployment in the field. Insight into the strain-specific nature of the protective efficacy of a vaccine candidate could also inform further optimization of the vaccine formulation prior to costly field studies (77,

78). It was only recently that a *P. falciparum* vaccine candidate, PfSPZ (a radiation-attenuated sporozoite vaccine), demonstrated significant protection against challenge with both homologous and heterologous *P. falciparum* strains in a CHMI study, albeit in a small number of volunteers (79, 80). The protective efficacy of this vaccine candidate is currently being tested in field sites in Africa where malaria is endemic. The CPS immunization strategy has also been shown to induce limited strain-transcending immunity (81).

Currently, only a limited number of defined *P. falciparum* strains are available for/have been used in CHMI: *P. falciparum* NF54 (an isolate of West African origin) (82), 3D7 (a clonal line derived from NF54) (65), 7G8 (a cloned line of the Brazilian IMTM22 isolate) (83), NF135.C10 (a clone derived from a Cambodian isolate) (84), and HMP02 (an isolate from Ghana) (23), with the latter available only for blood-stage challenge. Although they originate from different geographical areas, it is unknown how representative these strains are of the antigenically diverse circulating strains in all areas of malaria endemicity. For non-*P. falciparum* species, limited work has been undertaken with *P. vivax* (as indicated above). The ability to access suitable *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi* isolates would also increase the value and utility of the CHMI model.

A recent perspectives paper from the U.S. Food and Drug Administration (FDA) discussed the possibility of using efficacy results from CHMI studies to support licensure of a malaria vaccine for use in travelers (85). Demonstrating breadth of protection against diverse strains would be critical for this, thus emphasizing the importance of further developing and characterizing different parasite strains for CHMI studies. This is not a straightforward undertaking. Initial considerations include having the necessary ethical approvals and the logistics of identifying suitable individuals for collection of *Plasmodium*-infected blood. The development process for the *P. falciparum* NF135.C10 clone involved four qualification criteria (84). They were that the strain (i) must consistently produce gametocytes and sporozoites (this is not relevant to isolates being developed for blood-stage CHMI), (ii) should be cloned to create a genetically homogeneous parasite population, (iii) must have sensitivity to commonly used antimalarials, and (iv) should be geographically and genetically distinct from the NF54 strain. Screening of $>$70 strains was required to eventually identify the NF135.C10 clone (86). For isolates being developed for blood-stage CHMI, cryopreserved *ex vivo* blood-stage parasite banks are finite resources. While cultured blood-stage parasite banks are therefore advantageous, not all *P. falciparum* isolates are easily culture adapted, and some of the non-*P. falciparum* species are not amenable to the large-scale culture that is required to manufacture blood-stage parasite banks.

Standardization of methodologies between different research centers. The standardization of methodologies and sharing of reagents are essential to enable a direct comparison of data generated across multiple study centers (63). This encompasses processes for the manufacturing and handling of the CHMI product, as well as assays that are used to determine initiation of rescue drug treatment and evaluate study efficacy endpoints, e.g., parasite detection methods and immunogenicity assays.

For parasite detection, Giemsa-stained thick blood smear microscopy has traditionally been the "gold standard" for CHMI studies, and in sporozoite-initiated CHMI, drug treatment is initiated as soon as parasites are detected to minimize adverse events and potential complications. Standardized reading of blood smears is essential for comparison of trial endpoints across different research centers (54). Nucleic acid tests (NATs) such as quantitative PCR (qPCR) and quantitative reverse transcriptase PCR (qRT-PCR) are also being increasingly used in both vaccine and drug evaluation CHMI studies (87). DNA-based NATs can also detect transiently circulating, dead parasites in the peripheral blood, resulting in a short period of false-positive results, while RNA-based NATs can have greater sensitivity. For many of the sporozoite-initiated CHMI studies, qPCR is only used for retrospective analysis to estimate PMR and liver load through statistical modeling (88, 89). For mosquito-bite initiated CHMI studies, it has been shown that the

use of PCR assays allows for quantitative measurement of parasitemia on average 3.5 days earlier than microscopy and increases the statistical power of CHMI to evaluate vaccine and drug efficacy (1). It has been suggested that using qPCR as a primary endpoint in the sporozoite-initiated CHMI has a number of advantages, including shortening the duration of parasitemia (prepatent period), which has the potential to reduce the number of clinical symptoms in the volunteers (1). It has been shown that this can be implemented without negatively impacting the evaluation of the protective efficacy of preerythrocytic vaccines (1). Studies involving induced blood-stage malaria infection have used qPCR as the primary outcome variable (90) or in combination with microscopy (13). For a NAT to be used to define efficacy outcomes in CHMI, the assay requires validation prior to use in challenge studies (85). A standardized and validated NAT, including the blood collection schedule, should also be employed across multiple study sites to facilitate comparison of study results, and this is particularly pertinent to modeling of the PMR (87).

Establishment of the CHMI models in areas where malaria is endemic. It has been suggested that early-phase and challenge studies utilizing CHMI models should be established in multiple sites in areas of malaria endemicity to increase the international capacity to conduct studies that would eventually support product licensure (91). There are a number of advantages to conducting these studies in areas of endemicity, including the following: capacity building in developing countries, study participants having the same genetic background as the eventual target population, and the possibility of examining the effect of prior malaria exposure and immunity on vaccine efficacy and thus potentially having a longer time period in which to observe vaccine efficacy before initiation of drug treatment due to the presence of preexisting immunity.

As outlined above, mosquito bite-initiated CHMI for *P. vivax* has been established in Colombia (69), for practical and logistical reasons. More recently, CHMI using *P. falciparum* cryopreserved sporozoites has been established in different sites in Africa (57, 64). The advantages of using cryopreserved parasites (sporozoites or blood-stage parasites) are pertinent to establishing this research capacity in areas of endemicity (and to CHMI generally): they can be transported and stored in a liquid nitrogen vapor phase, and the administration of a predefined number of parasites would be associated with a reduction in site-to-site and trial-to-trial variation if standardized procedures are used.

There are many additional factors that must be considered when conducting CHMI studies in malaria-exposed individuals in areas of malaria endemicity. These include the following: ensuring that there are adequate clinical and laboratory resources with appropriately trained staff, dealing with a multitiered system of ethical review, defining appropriate levels of monetary compensation for study participation, ensuring comprehension of the research by participants, accounting for possible confounding of efficacy data by hemoglobinopathies, and grouping participants according to prior exposure (there are no validated assays for this), as naturally acquired immunity may impact PMR (57). Considerable research will be required to characterize the interaction between injected parasites (sporozoites and blood-stage parasites) and preexisting naturally acquired immune responses, as this will need to be factored into CHMI study design in areas of endemicity. The possibility of natural transmission of the challenge parasite strain to local areas also needs to be considered in relation to gametocyte appearance in the blood and drug treatment initiation time points.

CONCLUSION

CHMI is a versatile clinical tool which can be employed in different ways: as an immunization strategy, to assess antimalarial drug and vaccine efficacy, and to elucidate aspects of the human immune response to the malaria parasite, disease processes, and malaria parasite biology. Recent advances and development of CHMI-specific reagents that are easily transferable between different research centers highlight the potential of this model to accelerate malaria vaccine and drug development as well as

a greater understanding of host-parasite interactions. There are a number of key scientific gaps which need to be addressed to enable a more comprehensive use of this model, including development and validation of non-*P. falciparum* human malaria parasite species for sporozoite and blood-stage CHMI and a greater availability of geographically and genetically distinct *Plasmodium* species and strains for vaccine and drug evaluation. The development of aseptic, purified cryopreserved sporozoites for different *Plasmodium* species and strains would increase the international capability to use the CHMI model for vaccine and drug efficacy testing. The establishment and greater utilization of this model in multiple research centers worldwide introduces new challenges and emphasizes the need for greater harmonization and standardization of CHMI-specific processes to enable direct comparison of data across these sites.

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REFERENCES

1. Walk J, Schats R, Langenberg MC, Reuling IJ, Teelen K, Roestenberg M, Hermsen CC, Visser LG, Sauerwein RW. 2016. Diagnosis and treatment based on quantitative PCR after controlled human malaria infection. Malar J 15:398. https://doi.org/10.1186/s12936-016-1434-z.
2. Austin SC, Stolley PD, Lasky T. 1992. The history of malariotherapy for neurosyphilis. Modern parallels. JAMA 268:516–519.
3. Snounou G, Perignon JL. 2013. Malariotherapy—insanity at the service of malariology. Adv Parasitol 81:223–255. https://doi.org/10.1016/B978-0-12-407826-0.00006-0.
4. Nierengarten MB. 2003. Malariotherapy to treat HIV patients? Lancet Infect Dis 3:321.
5. Coatney HR, Cooper WC, Ruhe DS, Young MD. 1949. Studies in human malaria; trials of quinacrine, colchicine (SN 12,080) and quinine against Chesson strain vivax malaria. Am J Hyg (Lond) 50:194–199.
6. Pombo DJ, Lawrence G, Hirunpetcharat C, Rzepczyk C, Bryden M, Cloonan N, Anderson K, Mahakunkijcharoen Y, Martin LB, Wilson D, Elliott S, Eisen DP, Weinberg JB, Saul A, Good MF. 2002. Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. Lancet 360:610–617. https://doi.org/10.1016/S0140-6736(02)09784-2.
7. Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ, van de Vegte-Bolmer M, van Schaijk B, Teelen K, Arens T, Spaarman L, de Mast Q, Roeffen W, Snounou G, Renia L, van der Ven A, Hermsen CC, Sauerwein R. 2009. Protection against a malaria challenge by sporozoite inoculation. N Engl J Med 361:468–477. https://doi.org/10.1056/NEJMoa0805832.
8. Mordmuller B, Surat G, Lagler H, Chakravarty S, Ishizuka AS, Lalremruata A, Gmeiner M, Campo JJ, Esen M, Ruben AJ, Held J, Calle CL, Mengue JB, Gebru T, Ibanez J, Sulyok M, James ER, Billingsley PF, Natasha KC, Manoj A, Murshedkar T, Gunasekera A, Eappen AG, Li T, Stafford RE, Li M, Felgner PL, Seder RA, Richie TL, Sim BK, Hoffman SL, Kremsner PG. 2017. Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. Nature 542:445–449. https://doi.org/10.1038/nature21060.
9. McCarthy JS, Baker M, O'Rourke P, Marquart L, Griffin P, Hooft van Huijsduijnen R, Mohrle JJ. 2016. Efficacy of OZ439 (artefenomel) against early *Plasmodium falciparum* blood-stage malaria infection in healthy volunteers. J Antimicrob Chemother 71:2620–2627. https://doi.org/10.1093/jac/dkw174.
10. McCarthy JS, Ruckle T, Djeriou E, Cantalloube C, Ter-Minassian D, Baker M, O'Rourke P, Griffin P, Marquart L, Hooft van Huijsduijnen R, Mohrle JJ. 2016. A phase II pilot trial to evaluate safety and efficacy of ferroquine against early *Plasmodium falciparum* in an induced blood-stage malaria infection study. Malar J 15:469. https://doi.org/10.1186/s12936-016-1511-3.
11. McCarthy JS, Sekuloski S, Griffin PM, Elliott S, Douglas N, Peatey C, Rockett R, O'Rourke P, Marquart L, Hermsen C, Duparc S, Mohrle J, Trenholme KR, Humberstone AJ. 2011. A pilot randomised trial of induced blood-stage Plasmodium falciparum infections in healthy volunteers for testing efficacy of new antimalarial drugs. PLoS One 6:e21914. https://doi.org/10.1371/journal.pone.0021914.
12. Nyunt MM, Hendrix CW, Bakshi RP, Kumar N, Shapiro TA. 2009. Phase I/II evaluation of the prophylactic antimalarial activity of pafuramidine in healthy volunteers challenged with *Plasmodium falciparum* sporozoites. Am J Trop Med Hyg 80:528–535.
13. Payne RO, Milne KH, Elias SC, Edwards NJ, Douglas AD, Brown RE, Silk SE, Biswas S, Miura K, Roberts R, Rampling TW, Venkatraman N, Hodgson SH, Labbe GM, Halstead FD, Poulton ID, Nugent FL, de Graaf H, Sukhtankar P, Williams NC, Ockenhouse CF, Kathcart AK, Qabar AN, Waters NC, Soisson LA, Birkett AJ, Cooke GS, Faust SN, Woods C, Ivinson K, McCarthy JS, Diggs CL, Vekemans J, Long CA, Hill AV, Lawrie AM, Dutta S, Draper SJ. 2016. Demonstration of the blood-stage *Plasmodium falciparum* controlled human malaria infection model to assess efficacy of the *P. falciparum* apical membrane antigen 1 vaccine, FMP21/AS01. J Infect Dis 213:1743–1751. https://doi.org/10.1093/infdis/jiw039.
14. Spring MD, Cummings JF, Ockenhouse CF, Dutta S, Reidler R, Angov E, Bergmann-Leitner E, Stewart VA, Bittner S, Juompan L, Kortepeter MG, Nielsen R, Krzych U, Tierney E, Ware LA, Dowler M, Hermsen CC, Sauerwein RW, de Vlas SJ, Ofori-Anyinam O, Lanar DE, Williams JL, Kester KE, Tucker K, Shi M, Malkin E, Long C, Diggs CL, Soisson L, Dubois MC, Ballou WR, Cohen J, Heppner DG, Jr. 2009. Phase 1/2a study of the malaria vaccine candidate apical membrane antigen-1 (AMA-1) administered in adjuvant system AS01B or AS02A. PLoS One 4:e5254. https://doi.org/10.1371/journal.pone.0005254.
15. Bennett JW, Yadava A, Tosh D, Sattabongkot J, Komisar J, Ware LA, McCarthy WF, Cowden JJ, Regules J, Spring MD, Paolino K, Hartzell JD, Cummings JF, Richie TL, Lumsden J, Kamau E, Murphy J, Lee C, Parekh F, Birkett A, Cohen J, Ballou WR, Polhemus ME, Vanloubbeeck YF, Vekemans J, Ockenhouse CF. 2016. Phase 1/2a trial of *Plasmodium vivax* malaria vaccine candidate VMP001/AS01B in malaria-naive adults: safety, immunogenicity, and efficacy. PLoS Negl Trop Dis 10:e0004423. https://doi.org/10.1371/journal.pntd.0004423.
16. Lawrence G, Cheng QQ, Reed C, Taylor D, Stowers A, Cloonan N, Rzepczyk C, Smillie A, Anderson K, Pombo D, Allworth A, Eisen D, Anders R, Saul A. 2000. Effect of vaccination with 3 recombinant asexual-stage malaria antigens on initial growth rates of *Plasmodium falciparum* in non-immune volunteers. Vaccine 18:1925–1931. https://doi.org/10.1016/S0264-410X(99)00444-2.
17. Arevalo-Herrera M, Vasquez-Jimenez JM, Lopez-Perez M, Vallejo AF, Amado-Garavito AB, Cespedes N, Castellanos A, Molina K, Trejos J, Onate J, Epstein JE, Richie TL, Herrera S. 2016. Protective efficacy of *Plasmodium vivax* radiation-attenuated sporozoites in Colombian volunteers: a randomized controlled trial. PLoS Negl Trop Dis 10:e0005070. https://doi.org/10.1371/journal.pntd.0005070.
18. Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, Holman LA, James ER, Billingsley PF, Gunasekera A, Richman A, Chakravarty S, Manoj A, Velmurugan S, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Plummer SH, Hendel CS, Novik L, Costner PJ, Mendoza FH,

Saunders JG, Nason MC, Richardson JH, Murphy J, Davidson SA, Richie TL, Sedegah M, Sutamihardja A, Fahle GA, Lyke KE, Laurens MB, Roederer M, Tewari K, Epstein JE, Sim BK, Ledgerwood JE, Graham BS, Hoffman SL. 2013. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science 341:1359–1365. https://doi.org/10.1126/science.1241800.

19. Duncan CJ, Sheehy SH, Ewer KJ, Douglas AD, Collins KA, Halstead FD, Elias SC, Lillie PJ, Rausch K, Aebig J, Miura K, Edwards NJ, Poulton ID, Hunt-Cooke A, Porter DW, Thompson FM, Rowland R, Draper SJ, Gilbert SC, Fay MP, Long CA, Zhu D, Wu Y, Martin LB, Anderson CF, Lawrie AM, Hill AV, Ellis RD. 2011. Impact on malaria parasite multiplication rates in infected volunteers of the protein-in-adjuvant vaccine AMA1-C1/Alhydrogel+CPG.7909. PLoS One 6:e22271. https://doi.org/10.1371/journal.pone.0022271.
20. Rampling T, Ewer KJ, Bowyer G, Bliss CM, Edwards NJ, Wright D, Payne RO, Venkatraman N, de Barra E, Snudden CM, Poulton ID, de Graaf H, Sukhtankar P, Roberts R, Ivinson K, Weltzin R, Rajkumar BY, Wille-Reece U, Lee CK, Ockenhouse CF, Sinden RE, Gerry S, Lawrie AM, Vekemans J, Morelle D, Lievens M, Ballou RW, Cooke GS, Faust SN, Gilbert S, Hill AV. 2016. Safety and high level efficacy of the combination malaria vaccine regimen of RTS,S/AS01B with chimpanzee adenovirus 63 and modified vaccinia Ankara vectored vaccines expressing ME-TRAP. J Infect Dis 214:772–781. https://doi.org/10.1093/infdis/jiw244.
21. Berna AZ, McCarthy JS, Wang RX, Saliba KJ, Bravo FG, Cassells J, Padovan B, Trowell SC. 2015. Analysis of breath specimens for biomarkers of *Plasmodium falciparum* infection. J Infect Dis 212:1120–1128. https://doi.org/10.1093/infdis/jiv176.
22. Beadle C, Long GW, Weiss WR, McElroy PD, Maret SM, Oloo AJ, Hoffman SL. 1994. Diagnosis of malaria by detection of *Plasmodium falciparum* HRP-2 antigen with a rapid dipstick antigen-capture assay. Lancet 343:564–568. https://doi.org/10.1016/S0140-6736(94)91520-2.
23. Stanisic DI, Gerrard J, Fink J, Griffin PM, Liu XQ, Sundac L, Sekuloski S, Rodriguez IB, Pingnet J, Yang Y, Zhou Y, Trenholme KR, Wang CY, Hackett H, Chan JA, Langer C, Hanssen E, Hoffman SL, Beeson JG, McCarthy JS, Good MF. 2016. Infectivity of *Plasmodium falciparum* in malaria-naive individuals is related to knob expression and cytoadherence of the parasite. Infect Immun 84:2689–2696. https://doi.org/10.1128/IAI.00414-16.
24. Dimonte S, Bruske EI, Hass J, Supan C, Salazar CL, Held J, Tschan S, Esen M, Flotenmeyer M, Koch I, Berger J, Bachmann A, Sim BK, Hoffman SL, Kremsner PG, Mordmuller B, Frank M. 2016. Sporozoite route of infection influences in vitro var gene transcription of *Plasmodium falciparum* parasites from controlled human infections. J Infect Dis 214:884–894. https://doi.org/10.1093/infdis/jiw225.
25. Bachmann A, Petter M, Krumkamp R, Esen M, Held J, Scholz JA, Li T, Sim BK, Hoffman SL, Kremsner PG, Mordmuller B, Duffy MF, Tannich E. 2016. Mosquito passage dramatically changes var gene expression in controlled human *Plasmodium falciparum* infections. PLoS Pathog 12:e1005538. https://doi.org/10.1371/journal.ppat.1005538.
26. Riedl J, Mordmuller B, Koder S, Pabinger I, Kremsner PG, Hoffman SL, Ramharter M, Ay C. 2016. Alterations of blood coagulation in controlled human malaria infection. Malar J 15:15. https://doi.org/10.1186/s12936-015-1079-3.
27. Woodberry T, Minigo G, Piera KA, Amante FH, Pinzon-Charry A, Good MF, Lopez JA, Engwerda CR, McCarthy JS, Anstey NM. 2012. Low-level *Plasmodium falciparum* blood-stage infection causes dendritic cell apoptosis and dysfunction in healthy volunteers. J Infect Dis 206:333–340. https://doi.org/10.1093/infdis/jis366.
28. Scholzen A, Teirlinck AC, Bijker EM, Roestenberg M, Hermsen CC, Hoffman SL, Sauerwein RW. 2014. BAFF and BAFF receptor levels correlate with B cell subset activation and redistribution in controlled human malaria infection. J Immunol 192:3719–3729. https://doi.org/10.4049/jimmunol.1302960.
29. Turner L, Wang CW, Lavstsen T, Mwakalinga SB, Sauerwein RW, Hermsen CC, Theander TG. 2011. Antibodies against PfEMP1, RIFIN, MSP3 and GLURP are acquired during controlled Plasmodium falciparum malaria infections in naive volunteers. PLoS One 6:e29025. https://doi.org/10.1371/journal.pone.0029025.
30. Montes de Oca M, Kumar R, Rivera FL, Amante FH, Sheel M, Faleiro RJ, Bunn PT, Best SE, Beattie L, Ng SS, Edwards CL, Boyle GM, Price RN, Anstey NM, Loughland JR, Burel J, Doolan DL, Haque A, McCarthy JS, Engwerda CR. 2016. Type I interferons regulate immune responses in humans with blood-stage *Plasmodium falciparum* infection. Cell Rep 17:399–412. https://doi.org/10.1016/j.celrep.2016.09.015.
31. Burel JG, Apte SH, Groves PL, Klein K, McCarthy JS, Doolan DL. 2016. Reduced Plasmodium parasite burden associates with CD38+ CD4+ T cells displaying cytolytic potential and impaired IFN-gamma production. PLoS Pathog 12:e1005839. https://doi.org/10.1371/journal.ppat.1005839.
32. Hodgson SH, Llewellyn D, Silk SE, Milne KH, Elias SC, Miura K, Kamuyu G, Juma EA, Magiri C, Muia A, Jin J, Spencer AJ, Longley RJ, Mercier T, Decosterd L, Long CA, Osier FH, Hoffman SL, Ogutu B, Hill AV, Marsh K, Draper SJ. 2016. Changes in serological immunology measures in UK and Kenyan adults post-controlled human malaria infection. Front Microbiol 7:1604.
33. Hastings IM, Kay K, Hodel EM. 2015. How robust are malaria parasite clearance rates as indicators of drug effectiveness and resistance? Antimicrob Agents Chemother 59:6428–6436. https://doi.org/10.1128/AAC.00481-15.
34. Sulyok M, Ruckle T, Roth A, Murbeth RE, Chalon S, Kerr N, Samec SS, Gobeau N, Calle CL, Ibanez J, Sulyok Z, Held J, Gebru T, Granados P, Bruckner S, Nguetse C, Mengue J, Lalremruata A, Sim KL, Hoffman SL, Mohrle JJ, Kremsner PG, Mordmuller B. 2017. DSM265 for *Plasmodium falciparum* chemoprophylaxis: a randomised, double blinded, phase 1 trial with controlled human malaria infection. Lancet Infect Dis https://doi.org/10.1016/S1473-3099(17)30139-1.
35. Sauerwein RW, Roestenberg M, Moorthy VS. 2011. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nat Rev Immunol 11:57–64. https://doi.org/10.1038/nri2902.
36. Thompson FM, Porter DW, Okitsu SL, Westerfeld N, Vogel D, Todryk S, Poulton I, Correa S, Hutchings C, Berthoud T, Dunachie S, Andrews L, Williams JL, Sinden R, Gilbert SC, Pluschke G, Zurbriggen R, Hill AV. 2008. Evidence of blood stage efficacy with a virosomal malaria vaccine in a phase IIa clinical trial. PLoS One 3:e1493. https://doi.org/10.1371/journal.pone.0001493.
37. Clyde DF. 1975. Immunization of man against falciparum and vivax malaria by use of attenuated sporozoites. Am J Trop Med Hyg 24:397–401. https://doi.org/10.4269/ajtmh.1975.24.397.
38. Regules JA, Cicatelli SB, Bennett JW, Paolino KM, Twomey PS, Moon JE, Kathcart AK, Hauns KD, Komisar JL, Qabar AN, Davidson SA, Dutta S, Griffith ME, Magee CD, Wojnarski M, Livezey JR, Kress AT, Waterman PE, Jongert E, Wille-Reece U, Volkmuth W, Emerling D, Robinson WH, Lievens M, Morelle D, Lee CK, Yassin-Rajkumar B, Weltzin R, Cohen J, Paris RM, Waters NC, Birkett AJ, Kaslow DC, Ballou WR, Ockenhouse CF, Vekemans J. 2016. Fractional third and fourth dose of RTS,S/AS01 malaria candidate vaccine: a phase 2a controlled human malaria parasite infection and immunogenicity study. J Infect Dis 214:762–771. https://doi.org/10.1093/infdis/jiw237.
39. Casares S, Brumeanu TD, Richie TL. 2010. The RTS,S malaria vaccine. Vaccine 28:4880–4894. https://doi.org/10.1016/j.vaccine.2010.05.033.
40. Bejon P, Andrews L, Andersen RF, Dunachie S, Webster D, Walther M, Gilbert SC, Peto T, Hill AV. 2005. Calculation of liver-to-blood inocula, parasite growth rates, and preerythrocytic vaccine efficacy, from serial quantitative polymerase chain reaction studies of volunteers challenged with malaria sporozoites. J Infect Dis 191:619–626. https://doi.org/10.1086/427243.
41. Douglas AD, Edwards NJ, Duncan CJ, Thompson FM, Sheehy SH, O'Hara GA, Anagnostou N, Walther M, Webster DP, Dunachie SJ, Porter DW, Andrews L, Gilbert SC, Draper SJ, Hill AV, Bejon P. 2013. Comparison of modeling methods to determine liver-to-blood inocula and parasite multiplication rates during controlled human malaria infection. J Infect Dis 208:340–345. https://doi.org/10.1093/infdis/jit156.
42. Sheehy SH, Duncan CJ, Elias SC, Choudhary P, Biswas S, Halstead FD, Collins KA, Edwards NJ, Douglas AD, Anagnostou NA, Ewer KJ, Havelock T, Mahungu T, Bliss CM, Miura K, Poulton ID, Lillie PJ, Antrobus RD, Berrie E, Moyle S, Gantlett K, Colloca S, Cortese R, Long CA, Sinden RE, Gilbert SC, Lawrie AM, Doherty T, Faust SN, Nicosia A, Hill AV, Draper SJ. 2012. ChAd63-MVA-vectored blood-stage malaria vaccines targeting MSP1 and AMA1: assessment of efficacy against mosquito bite challenge in humans. Mol Ther 20:2355–2368. https://doi.org/10.1038/mt.2012.223.
43. Rosenberg R, Wirtz RA, Schneider I, Burge R. 1990. An estimation of the number of malaria sporozoites ejected by a feeding mosquito. Trans R Soc Trop Med Hyg 84:209–212. https://doi.org/10.1016/0035-9203(90)90258-G.
44. Ponnudurai T, Lensen AH, van Gemert GJ, Bolmer MG, Meuwissen JH. 1991. Feeding behaviour and sporozoite ejection by infected Anoph-

eles stephensi. Trans R Soc Trop Med Hyg 85:175–180. https://doi.org/10.1016/0035-9203(91)90012-N.
45. Sheehy SH, Spencer AJ, Douglas AD, Sim BK, Longley RJ, Edwards NJ, Poulton ID, Kimani D, Williams AR, Anagnostou NA, Roberts R, Kerridge S, Voysey M, James ER, Billingsley PF, Gunasekera A, Lawrie AM, Hoffman SL, Hill AV. 2013. Optimising controlled human malaria infection studies using cryopreserved parasites administered by needle and syringe. PLoS One 8:e65960. https://doi.org/10.1371/journal.pone.0065960.
46. Sanderson F, Andrews L, Douglas AD, Hunt-Cooke A, Bejon P, Hill AV. 2008. Blood-stage challenge for malaria vaccine efficacy trials: a pilot study with discussion of safety and potential value. Am J Trop Med Hyg 78:878–883.
47. Duncan CJ, Draper SJ. 2012. Controlled human blood stage malaria infection: current status and potential applications. Am J Trop Med Hyg 86:561–565. https://doi.org/10.4269/ajtmh.2012.11-0504.
48. Edstein MD, Kotecka BM, Anderson KL, Pombo DJ, Kyle DE, Rieckmann KH, Good MF. 2005. Lengthy antimalarial activity of atovaquone in human plasma following atovaquone-proguanil administration. Antimicrob Agents Chemother 49:4421–4422. https://doi.org/10.1128/AAC.49.10.4421-4422.2005.
49. Roestenberg M, Teirlinck AC, McCall MB, Teelen K, Makamdop KN, Wiersma J, Arens T, Beckers P, van Gemert G, van de Vegte-Bolmer M, van der Ven AJ, Luty AJ, Hermsen CC, Sauerwein RW. 2011. Long-term protection against malaria after experimental sporozoite inoculation: an open-label follow-up study. Lancet 377:1770–1776. https://doi.org/10.1016/S0140-6736(11)60360-7.
50. Bijker EM, Bastiaens GJ, Teirlinck AC, van Gemert GJ, Graumans W, van de Vegte-Bolmer M, Siebelink-Stoter R, Arens T, Teelen K, Nahrendorf W, Remarque EJ, Roeffen W, Jansens A, Zimmerman D, Vos M, van Schaijk BC, Wiersma J, van der Ven AJ, de Mast Q, van Lieshout L, Verweij JJ, Hermsen CC, Scholzen A, Sauerwein RW. 2013. Protection against malaria after immunization by chloroquine prophylaxis and sporozoites is mediated by preerythrocytic immunity. Proc Natl Acad Sci U S A 110:7862–7867. https://doi.org/10.1073/pnas.1220360110.
51. Bijker EM, Schats R, Obiero JM, Behet MC, van Gemert GJ, van de Vegte-Bolmer M, Graumans W, van Lieshout L, Bastiaens GJ, Teelen K, Hermsen CC, Scholzen A, Visser LG, Sauerwein RW. 2014. Sporozoite immunization of human volunteers under mefloquine prophylaxis is safe, immunogenic and protective: a double-blind randomized controlled clinical trial. PLoS One 9:e112910. https://doi.org/10.1371/journal.pone.0112910.
52. Kublin JG, Mikolajczak SA, Sack BK, Fishbaugher ME, Seilie A, Shelton L, VonGoedert T, Firat M, Magee S, Fritzen E, Betz W, Kain HS, Dankwa DA, Steel RW, Vaughan AM, Noah Sather D, Murphy SC, Kappe SH. 2017. Complete attenuation of genetically engineered *Plasmodium falciparum* sporozoites in human subjects. Sci Transl Med 9:eaad9099. https://doi.org/10.1126/scitranslmed.aad9099.
53. Stanisic DI, Good MF. 2015. Whole organism blood stage vaccines against malaria. Vaccine 33:7469–7475. https://doi.org/10.1016/j.vaccine.2015.09.057.
54. Laurens MB, Duncan CJ, Epstein JE, Hill AV, Komisar JL, Lyke KE, Ockenhouse CF, Richie TL, Roestenberg M, Sauerwein RW, Spring MD, Talley AK, Moorthy VS, Consensus Group on Design of Clinical Trials of Controlled Human Malaria Infection. 2012. A consultation on the optimization of controlled human malaria infection by mosquito bite for evaluation of candidate malaria vaccines. Vaccine 30:5302–5304. https://doi.org/10.1016/j.vaccine.2012.04.088.
55. Laurens MB, Billingsley P, Richman A, Eappen AG, Adams M, Li T, Chakravarty S, Gunasekera A, Jacob CG, Sim BK, Edelman R, Plowe CV, Hoffman SL, Lyke KE. 2013. Successful human infection with *P. falciparum* using three aseptic Anopheles stephensi mosquitoes: a new model for controlled human malaria infection. PLoS One 8:e68969. https://doi.org/10.1371/journal.pone.0068969.
56. Roestenberg M, Bijker EM, Sim BK, Billingsley PF, James ER, Bastiaens GJ, Teirlinck AC, Scholzen A, Teelen K, Arens T, van der Ven AJ, Gunasekera A, Chakravarty S, Velmurugan S, Hermsen CC, Sauerwein RW, Hoffman SL. 2013. Controlled human malaria infections by intradermal injection of cryopreserved *Plasmodium falciparum* sporozoites. Am J Trop Med Hyg 88:5–13. https://doi.org/10.4269/ajtmh.2012.12-0613.
57. Hodgson SH, Juma E, Salim A, Magiri C, Kimani D, Njenga D, Muia A, Cole AO, Ogwang C, Awuondo K, Lowe B, Munene M, Billingsley PF, James ER, Gunasekera A, Sim BK, Njuguna P, Rampling TW, Richman A, Abebe Y, Kamuyu G, Muthui M, Elias SC, Molyneux S, Gerry S, Macharia A, Williams TN, Bull PC, Hill AV, Osier FH, Draper SJ, Bejon P, Hoffman SL, Ogutu B, Marsh K. 2014. Evaluating controlled human malaria infection in Kenyan adults with varying degrees of prior exposure to *Plasmodium falciparum* using sporozoites administered by intramuscular injection. Front Microbiol 5:686. https://doi.org/10.3389/fmicb.2014.00686.
58. Gomez-Perez GP, Legarda A, Munoz J, Sim BK, Ballester MR, Dobano C, Moncunill G, Campo JJ, Cistero P, Jimenez A, Barrios D, Mordmuller B, Pardos J, Navarro M, Zita CJ, Nhamuave CA, Garcia-Basteiro AL, Sanz A, Aldea M, Manoj A, Gunasekera A, Billingsley PF, Aponte JJ, James ER, Guinovart C, Antonijoan RM, Kremsner PG, Hoffman SL, Alonso PL. 2015. Controlled human malaria infection by intramuscular and direct venous inoculation of cryopreserved *Plasmodium falciparum* sporozoites in malaria-naive volunteers: effect of injection volume and dose on infectivity rates. Malar J 14:306. https://doi.org/10.1186/s12936-015-0817-x.
59. Mordmuller B, Supan C, Sim KL, Gomez-Perez GP, Ospina Salazar CL, Held J, Bolte S, Esen M, Tschan S, Joanny F, Lamsfus Calle C, Lohr SJ, Lalremruata A, Gunasekera A, James ER, Billingsley PF, Richman A, Chakravarty S, Legarda A, Munoz J, Antonijoan RM, Ballester MR, Hoffman SL, Alonso PL, Kremsner PG. 2015. Direct venous inoculation of *Plasmodium falciparum* sporozoites for controlled human malaria infection: a dose-finding trial in two centres. Malar J 14:117. https://doi.org/10.1186/s12936-015-0628-0.
60. Epstein JE, Rao S, Williams F, Freilich D, Luke T, Sedegah M, de la Vega P, Sacci J, Richie TL, Hoffman SL. 2007. Safety and clinical outcome of experimental challenge of human volunteers with *Plasmodium falciparum*-infected mosquitoes: an update. J Infect Dis 196:145–154. https://doi.org/10.1086/518510.
61. Garver LS, Dowler M, Davidson SA. 2015. Controlled human malaria infection at the Walter Reed Army Institute of Research: the past, present, and future from an entomological perspective. US Army Med Dep J 2015:16–24.
62. Verhage DF, Telgt DS, Bousema JT, Hermsen CC, van Gemert GJ, van der Meer JW, Sauerwein RW. 2005. Clinical outcome of experimental human malaria induced by *Plasmodium falciparum*-infected mosquitoes. Neth J Med 63:52–58.
63. Roestenberg M, O'Hara GA, Duncan CJ, Epstein JE, Edwards NJ, Scholzen A, van der Ven AJ, Hermsen CC, Hill AV, Sauerwein RW. 2012. Comparison of clinical and parasitological data from controlled human malaria infection trials. PLoS One 7:e38434. https://doi.org/10.1371/journal.pone.0038434.
64. Shekalaghe S, Rutaihwa M, Billingsley PF, Chemba M, Daubenberger CA, James ER, Mpina M, Ali Juma O, Schindler T, Huber E, Gunasekera A, Manoj A, Simon B, Saverino E, Church LW, Hermsen CC, Sauerwein RW, Plowe C, Venkatesan M, Sasi P, Lweno O, Mutani P, Hamad A, Mohammed A, Urassa A, Mzee T, Padilla D, Ruben A, Sim BK, Tanner M, Abdulla S, Hoffman SL. 2014. Controlled human malaria infection of Tanzanians by intradermal injection of aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites. Am J Trop Med Hyg 91:471–480. https://doi.org/10.4269/ajtmh.14-0119.
65. Cheng Q, Lawrence G, Reed C, Stowers A, Ranford-Cartwright L, Creasey A, Carter R, Saul A. 1997. Measurement of *Plasmodium falciparum* growth rates in vivo: a test of malaria vaccines. Am J Trop Med Hyg 57:495–500. https://doi.org/10.4269/ajtmh.1997.57.495.
66. Stanisic DI, Liu XQ, De SL, Batzloff MR, Forbes T, Davis CB, Sekuloski S, Chavchich M, Chung W, Trenholme K, McCarthy JS, Li T, Sim BK, Hoffman SL, Good MF. 2015. Development of cultured *Plasmodium falciparum* blood-stage malaria cell banks for early phase in vivo clinical trial assessment of anti-malaria drugs and vaccines. Malar J 14:143. https://doi.org/10.1186/s12936-015-0663-x.
67. McCarthy JS, Griffin PM, Sekuloski S, Bright AT, Rockett R, Looke D, Elliott S, Whiley D, Sloots T, Winzeler EA, Trenholme KR. 2013. Experimentally induced blood-stage *Plasmodium vivax* infection in healthy volunteers. J Infect Dis 208:1688–1694. https://doi.org/10.1093/infdis/jit394.
68. Herrera S, Solarte Y, Jordan-Villegas A, Echavarria JF, Rocha L, Palacios R, Ramirez O, Velez JD, Epstein JE, Richie TL, Arevalo-Herrera M. 2011. Consistent safety and infectivity in sporozoite challenge model of *Plasmodium vivax* in malaria-naive human volunteers. Am J Trop Med Hyg 84:4–11. https://doi.org/10.4269/ajtmh.2011.09-0498.
69. Arevalo-Herrera M, Forero-Pena DA, Rubiano K, Gomez-Hincapie J, Martinez NL, Lopez-Perez M, Castellanos A, Cespedes N, Palacios R,

Onate JM, Herrera S. 2014. *Plasmodium vivax* sporozoite challenge in malaria-naive and semi-immune Colombian volunteers. PLoS One 9:e99754. https://doi.org/10.1371/journal.pone.0099754.
70. Herrera S, Fernandez O, Manzano MR, Murrain B, Vergara J, Blanco P, Palacios R, Velez JD, Epstein JE, Chen-Mok M, Reed ZH, Arevalo-Herrera M. 2009. Successful sporozoite challenge model in human volunteers with *Plasmodium vivax* strain derived from human donors. Am J Trop Med Hyg 81:740–746. https://doi.org/10.4269/ajtmh.2009.09-0194.
71. Payne RO, Griffin PM, McCarthy JS, Draper SJ. 2017. *Plasmodium vivax* controlled human malaria infection—progress and prospects. Trends Parasitol 33:141–150. https://doi.org/10.1016/j.pt.2016.11.001.
72. Bennett JW, Pybus BS, Yadava A, Tosh D, Sousa JC, McCarthy WF, Deye G, Melendez V, Ockenhouse CF. 2013. Primaquine failure and cytochrome P-450 2D6 in *Plasmodium vivax* malaria. N Engl J Med 369:1381–1382. https://doi.org/10.1056/NEJMc1301936.
73. Marcsisin SR, Reichard G, Pybus BS. 2016. Primaquine pharmacology in the context of CYP 2D6 pharmacogenomics: current state of the art. Pharmacol Ther 161:1–10. https://doi.org/10.1016/j.pharmthera.2016.03.011.
74. Griffin P, Pasay C, Elliott S, Sekuloski S, Sikulu M, Hugo L, Khoury D, Cromer D, Davenport M, Sattabongkot J, Ivinson K, Ockenhouse C, McCarthy J. 2016. Safety and reproducibility of a clinical trial system using induced blood stage *Plasmodium vivax* infection and its potential as a model to evaluate malaria transmission. PLoS Negl Trop Dis 10:e0005139. https://doi.org/10.1371/journal.pntd.0005139.
75. RTS,S Clinical Trials Partnership, Agnandji ST, Lell B, Soulanoudjingar SS, Fernandes JF, Abossolo BP, Conzelmann C, Methogo BG, Doucka Y, Flamen A, Mordmuller B, Issifou S, Kremsner PG, Sacarlal J, Aide P, Lanaspa M, Aponte JJ, Nhamuave A, Quelhas D, Bassat Q, Mandjate S, Macete E, Alonso P, Abdulla S, Salim N, Juma O, Shomari M, Shubis K, Machera F, Hamad AS, Minja R, Mtoro A, Sykes A, Ahmed S, Urassa AM, Ali AM, Mwangoka G, Tanner M, Tinto H, D'Alessandro U, et al. 2011. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. N Engl J Med 365:1863–1875. https://doi.org/10.1056/NEJMoa1102287.
76. Neafsey DE, Juraska M, Bedford T, Benkeser D, Valim C, Griggs A, Lievens M, Abdulla S, Adjei S, Agbenyega T, Agnandji ST, Aide P, Anderson S, Ansong D, Aponte JJ, Asante KP, Bejon P, Birkett AJ, Bruls M, Connolly KM, D'Alessandro U, Dobano C, Gesase S, Greenwood B, Grimsby J, Tinto H, Hamel MJ, Hoffman I, Kamthunzi P, Kariuki S, Kremsner PG, Leach A, Lell B, Lennon NJ, Lusingu J, Marsh K, Martinson F, Molel JT, Moss EL, Njuguna P, Ockenhouse CF, Ogutu BR, Otieno W, Otieno L, Otieno K, Owusu-Agyei S, Park DJ, Pelle K, Robbins D, Russ C, Ryan EM, Sacarlal J, Sogoloff B, Sorgho H, Tanner M, Theander T, Valea I, Volkman SK, Yu Q, Lapierre D, Birren BW, Gilbert PB, Wirth DF. 2015. Genetic diversity and protective efficacy of the RTS,S/AS01 malaria vaccine. N Engl J Med 373:2025–2037. https://doi.org/10.1056/NEJMoa1505819.
77. Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK, Guindo AB, Traore K, Traore I, Kouriba B, Diallo DA, Diarra I, Daou M, Dolo A, Tolo Y, Sissoko MS, Niangaly A, Sissoko M, Takala-Harrison S, Lyke KE, Wu Y, Blackwelder WC, Godeaux O, Vekemans J, Dubois MC, Ballou WR, Cohen J, Thompson D, Dube T, Soisson L, Diggs CL, House B, Lanar DE, Dutta S, Heppner DG, Jr, Plowe CV. 2011. A field trial to assess a blood-stage malaria vaccine. N Engl J Med 365:1004–1013. https://doi.org/10.1056/NEJMoa1008115.
78. Genton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, Rare L, Baisor M, Lorry K, Brown GV, Pye D, Irving DO, Smith TA, Beck HP, Alpers MP. 2002. 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 185:820–827. https://doi.org/10.1086/339342.
79. Lyke KE, Ishizuka AS, Berry AA, Chakravarty S, DeZure A, Enama ME, James ER, Billingsley PF, Gunasekera A, Manoj A, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Kc N, Murshedkar T, Mendoza FH, Gordon IJ, Zephir KL, Holman LA, Plummer SH, Hendel CS, Novik L, Costner PJ, Saunders JG, Berkowitz NM, Flynn BJ, Nason MC, Garver LS, Laurens MB, Plowe CV, Richie TL, Graham BS, Roederer M, Sim BK, Ledgerwood JE, Hoffman SL, Seder RA. 2017. Attenuated PfSPZ Vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. Proc Natl Acad Sci U S A 114:2711–2716. https://doi.org/10.1073/pnas.1615324114.
80. Epstein JE, Paolino KM, Richie TL, Sedegah M, Singer A, Ruben AJ, Chakravarty S, Stafford A, Ruck RC, Eappen AG, Li T, Billingsley PF, Manoj A, Silva JC, Moser K, Nielsen R, Tosh D, Cicatelli S, Ganeshan H, Case J, Padilla D, Davidson S, Garver L, Saverino E, Murshedkar T, Gunasekera A, Twomey PS, Reyes S, Moon JE, James ER, Kc N, Li M, Abot E, Belmonte A, Hauns K, Belmonte M, Huang J, Vasquez C, Remich S, Carrington M, Abebe Y, Tillman A, Hickey B, Regules J, Villasante E, Sim BK, Hoffman SL. 2017. Protection against Plasmodium falciparum malaria by PfSPZ vaccine. JCI Insight 2:e89154. https://doi.org/10.1172/jci.insight.89154.
81. Schats R, Bijker EM, van Gemert GJ, Graumans W, van de Vegte-Bolmer M, van Lieshout L, Haks MC, Hermsen CC, Scholzen A, Visser LG, Sauerwein RW. 2015. Heterologous protection against malaria after immunization with *Plasmodium falciparum* sporozoites. PLoS One 10:e0124243. https://doi.org/10.1371/journal.pone.0124243.
82. Delemarre BJ, van der Kaay HJ. 1979. Tropical malaria contracted the natural way in the Netherlands. Ned Tijdschr Geneeskd 123:1981–1982.
83. Burkot TR, Williams JL, Schneider I. 1984. Infectivity to mosquitoes of *Plasmodium falciparum* clones grown in vitro from the same isolate. Trans R Soc Trop Med Hyg 78:339–341. https://doi.org/10.1016/0035-9203(84)90114-7.
84. Teirlinck AC, Roestenberg M, van de Vegte-Bolmer M, Scholzen A, Heinrichs MJ, Siebelink-Stoter R, Graumans W, van Gemert GJ, Teelen K, Vos MW, Nganou-Makamdop K, Borrmann S, Rozier YP, Erkens MA, Luty AJ, Hermsen CC, Sim BK, van Lieshout L, Hoffman SL, Visser LG, Sauerwein RW. 2013. NF135.C10: a new *Plasmodium falciparum* clone for controlled human malaria infections. J Infect Dis 207:656–660. https://doi.org/10.1093/infdis/jis725.
85. Chattopadhyay R, Pratt D. 2017. Role of controlled human malaria infection (CHMI) in malaria vaccine development: a U.S. Food & Drug Administration (FDA) perspective. Vaccine 35:2767–2769.
86. Bijker EM, Schats R, Visser LG, Sauerwein RW, Scholzen A. 2015. Ex vivo lymphocyte phenotyping during *Plasmodium falciparum* sporozoite immunization in humans. Parasite Immunol 37:590–598. https://doi.org/10.1111/pim.12276.
87. Murphy SC, Hermsen CC, Douglas AD, Edwards NJ, Petersen I, Fahle GA, Adams M, Berry AA, Billman ZP, Gilbert SC, Laurens MB, Leroy O, Lyke KE, Plowe CV, Seilie AM, Strauss KA, Teelen K, Hill AV, Sauerwein RW. 2014. External quality assurance of malaria nucleic acid testing for clinical trials and eradication surveillance. PLoS One 9:e97398. https://doi.org/10.1371/journal.pone.0097398.
88. Hermsen CC, de Vlas SJ, van Gemert GJ, Telgt DS, Verhage DF, Sauerwein RW. 2004. Testing vaccines in human experimental malaria: statistical analysis of parasitemia measured by a quantitative real-time polymerase chain reaction. Am J Trop Med Hyg 71:196–201.
89. Hermsen CC, Telgt DS, Linders EH, van de Locht LA, Eling WM, Mensink EJ, Sauerwein RW. 2001. Detection of *Plasmodium falciparum* malaria parasites in vivo by real-time quantitative PCR. Mol Biochem Parasitol 118:247–251. https://doi.org/10.1016/S0166-6851(01)00379-6.
90. Marquart L, Baker M, O'Rourke P, McCarthy JS. 2015. Evaluating the pharmacodynamic effect of antimalarial drugs in clinical trials by quantitative PCR. Antimicrob Agents Chemother 59:4249–4259. https://doi.org/10.1128/AAC.04942-14.
91. Chilengi R. 2009. Clinical development of malaria vaccines: should earlier trials be done in malaria endemic countries? Hum Vaccin 5:627–636. https://doi.org/10.4161/hv.9141.
92. Deye GA, Miller RS, Miller L, Salas CJ, Tosh D, Macareo L, Smith BL, Fracisco S, Clemens EG, Murphy J, Sousa JC, Dumler JS, Magill AJ. 2012. Prolonged protection provided by a single dose of atovaquone-proguanil for the chemoprophylaxis of *Plasmodium falciparum* malaria in a human challenge model. Clin Infect Dis 54:232–239. https://doi.org/10.1093/cid/cir770.
93. Sulyok M, Ruckle T, Roth A, Murbeth RE, Chalon S, Kerr N, Samec SS, Gobeau N, Calle CL, Ibanez J, Sulyok Z, Held J, Gebru T, Granados P, Bruckner S, Nguetse C, Mengue J, Lalremruata A, Sim BKL, Hoffman SL, Mohrle JJ, Kremsner PG, Mordmuller B. 2017. DSM265 for *Plasmodium falciparum* chemoprophylaxis: a randomised, double blinded, phase 1 trial with controlled human malaria infection. Lancet Infect Dis 17:636–644. https://doi.org/10.1016/S1473-3099(17)30139-1.
94. Berman JD, Nielsen R, Chulay JD, Dowler M, Kain KC, Kester KE, Williams J, Whelen AC, Shmuklarsky MJ. 2001. Causal prophylactic efficacy of atovaquone-proguanil (Malarone) in a human challenge model. Trans R Soc Trop Med Hyg 95:429–432. https://doi.org/10.1016/S0035-9203(01)90206-8.
95. Rieckmann KH, McNamara JV, Frischer H, Stockert TA, Carson PE, Powell RD. 1968. Gametocytocidal and sporontocidal effects of primaquine

and of sulfadiazine with pyrimethamine in a chloroquine-resistant strain of *Plasmodium falciparum*. Bull World Health Organ 38:625–632.
96. Trenholme CM, Williams RL, Desjardins RE, Frischer H, Carson PE, Rieckmann KH, Canfield CJ. 1975. Mefloquine (WR 142,490) in the treatment of human malaria. Science 190:792–794. https://doi.org/10.1126/science.1105787.
97. Rieckmann KH, Trenholme GM, Williams RL, Carson PE, Frischer H, Desjardins RE. 1974. Prophylactic activity of mefloquine hydrochloride (WR 142490) in drug-resistant malaria. Bull World Health Organ 51:375–377.
98. Martin DC, Arnold JD, Clyde DF, al-Ibrahim M, Carson PE, Rieckmann KH, Willerson D, Jr. 1973. A quinoline methanol (WR 30090) for treatment of acute malaria. Antimicrob Agents Chemother 3:214–219. https://doi.org/10.1128/AAC.3.2.214.
99. Arnold JD, Martin DC, Carson PE, Rieckmann KH, Willerson D, Jr, Clyde DF, Miller RM. 1973. A phenanthrene methanol (WR 33063) for treatment of acute malaria. Antimicrob Agents Chemother 3:207–213. https://doi.org/10.1128/AAC.3.2.207.
100. Rieckmann KH, Powell RD, McNamara JV, Willerson D, Jr, Lass L, Frischer H, Carson PE. 1971. Effects of tetracycline against chloroquine-resistant and chloroquine-sensitive *Plasmodium falciparum*. Am J Trop Med Hyg 20:811–815. https://doi.org/10.4269/ajtmh.1971.20.811.
101. Willerson D, Jr, Rieckmann KH, Carson PE, Frischer H. 1972. Effects of minocycline against chloroquine-resistant falciparum malaria. Am J Trop Med Hyg 21:857–862. https://doi.org/10.4269/ajtmh.1972.21.857.
102. Williams RL, Trenholme GM, Carson PE, Frischer H, Rieckmann KH. 1975. Acetylator phenotype and response of individuals infected with a chloroquine-resistant strain of *Plasmodium falciparum* to sulfalene and pyrimethamine. Am J Trop Med Hyg 24:734–739. https://doi.org/10.4269/ajtmh.1975.24.734.
103. Williams RL, Trenholme GM, Carson PE, Frischer H, Rieckmann KH. 1978. The influence of acetylator phenotype on the response to sulfalene in individuals with chloroquine-resistant falciparum malaria. Am J Trop Med Hyg 27:226–231. https://doi.org/10.4269/ajtmh.1978.27.226.
104. Willerson D, Jr, Rieckmann KH, Kass L, Carson PE, Frischer H, Bowman JE. 1972. The chemoprophylactic use of diformyl diaminodiphenyl sulfone (DFD) in falciparum malaria. Am J Trop Med Hyg 21:138–143. https://doi.org/10.4269/ajtmh.1972.21.138.
105. Rieckmann KH, McNamara JV, Kass L, Powell RD. 1969. Gametocytocidal and sporontocidal effects of primaquine upon two strains of *Plasmodium falciparum*. Mil Med 134:802–819.
106. McNamara JV, Rieckmann KH, Frischer H, Stockert TA, Carson PE, Powell RD. 1967. Acquired decrease in sensitivity to quinine observed during studies with a strain of chloroquine-resistant *Plasmodium falciparum*. Ann Trop Med Parasitol 61:386–395. https://doi.org/10.1080/00034983.1967.11686504.
107. Rinehart J, Arnold J, Canfield CJ. 1976. Evaluation of two phenanthrenemethanols for antimalarial activity in man: WR 122,455 and WR 171,669. Am J Trop Med Hyg 25:769–774. https://doi.org/10.4269/ajtmh.1976.25.769.
108. Cosgriff TM, Boudreau EF, Pamplin CL 3rd, Berman JD, Shmuklarsky MJ, Canfield CJ. 1984. Evaluation of the 4-pyridinemethanol WR 180,409 (enpiroline) in the treatment of induced *Plasmodium falciparum* infections in healthy, non-immune subjects. Am J Trop Med Hyg 33:767–771. https://doi.org/10.4269/ajtmh.1984.33.767.
109. Cosgriff TM, Boudreau EF, Pamplin CL, Doberstyn EB, Desjardins RE, Canfield CJ. 1982. Evaluation of the antimalarial activity of the phenanthrenemethanol halofantrine (WR 171,669). Am J Trop Med Hyg 31:1075–1079. https://doi.org/10.4269/ajtmh.1982.31.1075.
110. McCarthy JS, Lotharius J, Ruckle T, Chalon S, Phillips MA, Elliott S, Sekuloski S, Griffin P, Ng CL, Fidock DA, Marquart L, Williams NS, Gobeau N, Bebrevska L, Rosario M, Marsh K, Mohrle JJ. 2017. Safety, tolerability, pharmacokinetics, and activity of the novel long-acting antimalarial DSM265: a two-part first-in-human phase 1a/1b randomised study. Lancet Infect Dis 17:626–635. https://doi.org/10.1016/S1473-3099(17)30171-8.
111. Smith CM, Jerkovic A, Truong TT, Foote SJ, McCarthy JS, McMorran BJ. 2017. Griseofulvin impairs intraerythrocytic growth of *Plasmodium falciparum* through ferrochelatase inhibition but lacks activity in an experimental human infection study. Sci Rep 7:41975. https://doi.org/10.1038/srep41975.
112. Krause A, Dingemanse J, Mathis A, Marquart L, Mohrle JJ, McCarthy JS. 2016. Pharmacokinetic/pharmacodynamic modelling of the antimalarial effect of Actelion-451840 in an induced blood stage malaria study in healthy subjects. Br J Clin Pharmacol 82:412–421. https://doi.org/10.1111/bcp.12962.
113. Pasay CJ, Rockett R, Sekuloski S, Griffin P, Marquart L, Peatey C, Wang CY, O'Rourke P, Elliott S, Baker M, Mohrle JJ, McCarthy JS. 2016. Piperaquine monotherapy of drug-susceptible *Plasmodium falciparum* infection results in rapid clearance of parasitemia but is followed by the appearance of gametocytemia. J Infect Dis 214:105–113. https://doi.org/10.1093/infdis/jiw128.
114. Fairley NH. 1947. Sidelights on malaria in man obtained by subinoculation experiments. Trans R Soc Trop Med Hyg 40:621–676. https://doi.org/10.1016/0035-9203(47)90025-4.
115. Clyde DF, Most H, McCarthy VC, Vanderberg JP. 1973. Immunization of man against sporozite-induced falciparum malaria. Am J Med Sci 266:169–177. https://doi.org/10.1097/00000441-197309000-00002.
116. Dunachie SJ, Walther M, Epstein JE, Keating S, Berthoud T, Andrews L, Andersen RF, Bejon P, Goonetilleke N, Poulton I, Webster DP, Butcher G, Watkins K, Sinden RE, Levine GL, Richie TL, Schneider J, Kaslow D, Gilbert SC, Carucci DJ, Hill AV. 2006. A DNA prime-modified vaccinia virus Ankara boost vaccine encoding thrombospondin-related adhesion protein but not circumsporozoite protein partially protects healthy malaria-naive adults against *Plasmodium falciparum* sporozoite challenge. Infect Immun 74:5933–5942. https://doi.org/10.1128/IAI.00590-06.
117. Dunachie SJ, Walther M, Vuola JM, Webster DP, Keating SM, Berthoud T, Andrews L, Bejon P, Poulton I, Butcher G, Watkins K, Sinden RE, Leach A, Moris P, Tornieporth N, Schneider J, Dubovsky F, Tierney E, Williams J, Heppner DG, Jr, Gilbert SC, Cohen J, Hill AV. 2006. A clinical trial of prime-boost immunisation with the candidate malaria vaccines RTS,S/AS02A and MVA-CS. Vaccine 24:2850–2859. https://doi.org/10.1016/j.vaccine.2005.12.041.
118. Hodgson SH, Ewer KJ, Bliss CM, Edwards NJ, Rampling T, Anagnostou NA, de Barra E, Havelock T, Bowyer G, Poulton ID, de Cassan S, Longley R, Illingworth JJ, Douglas AD, Mange PB, Collins KA, Roberts R, Gerry S, Berrie E, Moyle S, Colloca S, Cortese R, Sinden RE, Gilbert SC, Bejon P, Lawrie AM, Nicosia A, Faust SN, Hill AV. 2015. Evaluation of the efficacy of ChAd63-MVA vectored vaccines expressing circumsporozoite protein and ME-TRAP against controlled human malaria infection in malaria-naive individuals. J Infect Dis 211:1076–1086. https://doi.org/10.1093/infdis/jiu579.
119. Hoffman SL, Goh LM, Luke TC, Schneider I, Le TP, Doolan DL, Sacci J, de la Vega P, Dowler M, Paul C, Gordon DM, Stoute JA, Church LW, Sedegah M, Heppner DG, Ballou WR, Richie TL. 2002. Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. J Infect Dis 185:1155–1164. https://doi.org/10.1086/339409.
120. McConkey SJ, Reece WH, Moorthy VS, Webster D, Dunachie S, Butcher G, Vuola JM, Blanchard TJ, Gothard P, Watkins K, Hannan CM, Everaere S, Brown K, Kester KE, Cummings J, Williams J, Heppner DG, Pathan A, Flanagan K, Arulanantham N, Roberts MT, Roy M, Smith GL, Schneider J, Peto T, Sinden RE, Gilbert SC, Hill AV. 2003. Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. Nat Med 9:729–735. https://doi.org/10.1038/nm881.
121. Ewer KJ, O'Hara GA, Duncan CJ, Collins KA, Sheehy SH, Reyes-Sandoval A, Goodman AL, Edwards NJ, Elias SC, Halstead FD, Longley RJ, Rowland R, Poulton ID, Draper SJ, Blagborough AM, Berrie E, Moyle S, Williams N, Siani L, Folgori A, Colloca S, Sinden RE, Lawrie AM, Cortese R, Gilbert SC, Nicosia A, Hill AV. 2013. Protective CD8+ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. Nat Commun 4:2836. https://doi.org/10.1038/ncomms3836.
122. Porter DW, Thompson FM, Berthoud TK, Hutchings CL, Andrews L, Biswas S, Poulton I, Prieur E, Correa S, Rowland R, Lang T, Williams J, Gilbert SC, Sinden RE, Todryk S, Hill AV. 2011. A human phase I/IIa malaria challenge trial of a polyprotein malaria vaccine. Vaccine 29:7514–7522. https://doi.org/10.1016/j.vaccine.2011.03.083.
123. Walther M, Thompson FM, Dunachie S, Keating S, Todryk S, Berthoud T, Andrews L, Andersen RF, Moore A, Gilbert SC, Poulton I, Dubovsky F, Tierney E, Correa S, Huntcooke A, Butcher G, Williams J, Sinden RE, Hill AV. 2006. Safety, immunogenicity, and efficacy of prime-boost immunization with recombinant poxvirus FP9 and modified vaccinia virus Ankara encoding the full-length *Plasmodium falciparum* circumsporozoite protein. Infect Immun 74:2706–2716. https://doi.org/10.1128/IAI.74.5.2706-2716.2006.
124. Webster DP, Dunachie S, Vuola JM, Berthoud T, Keating S, Laidlaw SM,

McConkey SJ, Poulton I, Andrews L, Andersen RF, Bejon P, Butcher G, Sinden R, Skinner MA, Gilbert SC, Hill AV. 2005. Enhanced T cell-mediated protection against malaria in human challenges by using the recombinant poxviruses FP9 and modified vaccinia virus Ankara. Proc Natl Acad Sci U S A 102:4836–4841. https://doi.org/10.1073/pnas.0406381102.

125. Ockenhouse CF, Sun PF, Lanar DE, Wellde BT, Hall BT, Kester K, Stoute JA, Magill A, Krzych U, Farley L, Wirtz RA, Sadoff JC, Kaslow DC, Kumar S, Church LW, Crutcher JM, Wizel B, Hoffman S, Lalvani A, Hill AV, Tine JA, Guito KP, de Taisne C, Anders R, Horii T, Paoletti E, Ballou WR. 1998. Phase I/IIa safety, immunogenicity, and efficacy trial of NYVAC-Pf7, a pox-vectored, multiantigen, multistage vaccine candidate for *Plasmodium falciparum* malaria. J Infect Dis 177:1664–1673. https://doi.org/10.1086/515331.
126. Chuang I, Sedegah M, Cicatelli S, Spring M, Polhemus M, Tamminga C, Patterson N, Guerrero M, Bennett JW, McGrath S, Ganeshan H, Belmonte M, Farooq F, Abot E, Banania JG, Huang J, Newcomer R, Rein L, Litilit D, Richie NO, Wood C, Murphy J, Sauerwein R, Hermsen CC, McCoy AJ, Kamau E, Cummings J, Komisar J, Sutamihardja A, Shi M, Epstein JE, Maiolatesi S, Tosh D, Limbach K, Angov E, Bergmann-Leitner E, Bruder JT, Doolan DL, King CR, Carucci D, Dutta S, Soisson L, Diggs C, Hollingdale MR, Ockenhouse CF, Richie TL. 2013. DNA prime/adenovirus boost malaria vaccine encoding *P. falciparum* CSP and AMA1 induces sterile protection associated with cell-mediated immunity. PLoS One 8:e55571. https://doi.org/10.1371/journal.pone.0055571.
127. Genton B, D'Acremont V, Lurati-Ruiz F, Verhage D, Audran R, Hermsen C, Wolters L, Reymond C, Spertini F, Sauerwein R. 2010. Randomized double-blind controlled phase I/IIa trial to assess the efficacy of malaria vaccine PfCS102 to protect against challenge with *P. falciparum*. Vaccine 28:6573–6580. https://doi.org/10.1016/j.vaccine.2010.07.067.
128. Hickey BW, Lumsden JM, Reyes S, Sedegah M, Hollingdale MR, Freilich DA, Luke TC, Charoenvit Y, Goh LM, Berzins MP, Bebris L, Sacci JB, Jr, De La Vega P, Wang R, Ganeshan H, Abot EN, Carucci DJ, Doolan DL, Brice GT, Kumar A, Aguiar J, Nutman TB, Leitman SF, Hoffman SL, Epstein JE, Richie TL. 2016. Mosquito bite immunization with radiation-attenuated *Plasmodium falciparum* sporozoites: safety, tolerability, protective efficacy and humoral immunogenicity. Malar J 15:377. https://doi.org/10.1186/s12936-016-1435-y.
129. Ishizuka AS, Lyke KE, DeZure A, Berry AA, Richie TL, Mendoza FH, Enama ME, Gordon IJ, Chang LJ, Sarwar UN, Zephir KL, Holman LA, James ER, Billingsley PF, Gunasekera A, Chakravarty S, Manoj A, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, K CN, Murshedkar T, DeCederfelt H, Plummer SH, Hendel CS, Novik L, Costner PJ, Saunders JG, Laurens MB, Plowe CV, Flynn B, Whalen WR, Todd JP, Noor J, Rao S, Sierra-Davidson K, Lynn GM, Epstein JE, Kemp MA, Fahle GA, Mikolajczak SA, Fishbaugher M, Sack BK, Kappe SH, Davidson SA, Garver LS, Bjorkstrom NK, Nason MC, Graham BS, Roederer M, Sim BK, Hoffman SL, Ledgerwood JE, Seder RA. 2016. Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. Nat Med 22:614–623. https://doi.org/10.1038/nm.4110.
130. Richie TL, Charoenvit Y, Wang R, Epstein JE, Hedstrom RC, Kumar S, Luke TC, Freilich DA, Aguiar JC, Sacci JB, Jr, Sedegah M, Nosek RA, Jr, De La Vega P, Berzins MP, Majam VF, Abot EN, Ganeshan H, Richie NO, Banania JG, Baraceros MF, Geter TG, Mere R, Bebris L, Limbach K, Hickey BW, Lanar DE, Ng J, Shi M, Hobart PM, Norman JA, Soisson LA, Hollingdale MR, Rogers WO, Doolan DL, Hoffman SL. 2012. Clinical trial in healthy malaria-naive adults to evaluate the safety, tolerability, immunogenicity and efficacy of MuStDO5, a five-gene, sporozoite/hepatic stage *Plasmodium falciparum* DNA vaccine combined with escalating dose human GM-CSF DNA. Hum Vaccin Immunother 8:1564–1584. https://doi.org/10.4161/hv.22129.
131. Tamminga C, Sedegah M, Maiolatesi S, Fedders C, Reyes S, Reyes A, Vasquez C, Alcorta Y, Chuang I, Spring M, Kavanaugh M, Ganeshan H, Huang J, Belmonte M, Abot E, Belmonte A, Banania J, Farooq F, Murphy J, Komisar J, Richie NO, Bennett J, Limbach K, Patterson NB, Bruder JT, Shi M, Miller E, Dutta S, Diggs C, Soisson LA, Hollingdale MR, Epstein JE, Richie TL. 2013. Human adenovirus 5-vectored *Plasmodium falciparum* NMRC-M3V-Ad-PfCA vaccine encoding CSP and AMA1 is safe, well-tolerated and immunogenic but does not protect against controlled human malaria infection. Hum Vaccin Immunother 9:2165–2177. https://doi.org/10.4161/hv.24941.
132. Ockenhouse CF, Regules J, Tosh D, Cowden J, Kathcart A, Cummings J, Paolino K, Moon J, Komisar J, Kamau E, Oliver T, Chhoeu A, Murphy J, Lyke K, Laurens M, Birkett A, Lee C, Weltzin R, Wille-Reece U, Sedegah M, Hendriks J, Versteege I, Pau MG, Sadoff J, Vanloubbeeck Y, Lievens M, Heerwegh D, Moris P, Guerra Mendoza Y, Jongert E, Cohen J, Voss G, Ballou WR, Vekemans J. 2015. Ad35.CS.01-RTS,S/AS01 heterologous prime boost vaccine efficacy against sporozoite challenge in healthy malaria-naive adults. PLoS One 10:e0131571. https://doi.org/10.1371/journal.pone.0131571.
133. Hoffman SL, Edelman R, Bryan JP, Schneider I, Davis J, Sedegah M, Gordon D, Church P, Gross M, Silverman C, Hollingdale M, Clyde D, Sztein M, Losonsky G, Paparello S, Jones TR. 1994. Safety, immunogenicity, and efficacy of a malaria sporozoite vaccine administered with monophosphoryl lipid A, cell wall skeleton of mycobacteria, and squalane as adjuvant. Am J Trop Med Hyg 51:603–612. https://doi.org/10.4269/ajtmh.1994.51.603.
134. Edelman R, Hoffman SL, Davis JR, Beier M, Sztein MB, Losonsky G, Herrington DA, Eddy HA, Hollingdale MR, Gordon DM, Clyde DF. 1993. Long-term persistence of sterile immunity in a volunteer immunized with X-irradiated *Plasmodium falciparum* sporozoites. J Infect Dis 168:1066–1070. https://doi.org/10.1093/infdis/168.4.1066.
135. Ballou WR, Sherwood JA, Neva FA, Gordon DM, Wirtz RA, Wasserman GF, Diggs CL, Hoffman SL, Hollingdale MR, Hockmeyer WT, Schneider I, Young JF, Reeve P, Chulay JD. 1987. Safety and efficacy of a recombinant DNA *Plasmodium falciparum* sporozoite vaccine. Lancet i:1277–1281.
136. Herrington D, Davis J, Nardin E, Beier M, Cortese J, Eddy H, Losonsky G, Hollingdale M, Sztein M, Levine M, Nussenzweig RS, Clyde D, Edelman R. 1991. Successful immunization of humans with irradiated malaria sporozoites: humoral and cellular responses of the protected individuals. Am J Trop Med Hyg 45:539–547. https://doi.org/10.4269/ajtmh.1991.45.539.
137. Herrington DA, Clyde DF, Davis JR, Baqar S, Murphy JR, Cortese JF, Bank RS, Nardin E, DiJohn D, Nussenzweig RS, Nussenzweig V, Torres JR, Murillo J, Cortesia M, Sturchler D, Hollingdale M, Levine MM. 1990. Human studies with synthetic peptide sporozoite vaccine (NANP)3-TT and immunization with irradiated sporozoites. Bull World Health Organ 68(Suppl):33–37.
138. Kester KE, Gray Heppner D, Jr, Moris P, Ofori-Anyinam O, Krzych U, Tornieporth N, McKinney D, Delchambre M, Ockenhouse CF, Voss G, Holland C, Beckey JP, Ballou WR, Cohen J, RTS,S/TRAP Group. 2014. Sequential phase 1 and phase 2 randomized, controlled trials of the safety, immunogenicity and efficacy of combined pre-erythrocytic vaccine antigens RTS,S and TRAP formulated with AS02 adjuvant system in healthy, malaria naive adults. Vaccine 32:6683–6691. https://doi.org/10.1016/j.vaccine.2014.06.033.
139. Stoute J, Kester K, Krzych U, Wellde B, Hall T, White K, Glenn G, Ockenhouse C, Garcon N, Schwenk R, Lanar D, Sun P, Momin P, Wirtz R, Golenda C, Slaoui M, Wortmann G, Holland C, Dowler M, Cohen J, Ballou W. 1998. Long-term efficacy and immune responses following immunization with the RTS,S malaria vaccine. The J Infect Dis 178:1139–1144. https://doi.org/10.1086/515657.
140. Cummings JF, Spring MD, Schwenk RJ, Ockenhouse CF, Kester KE, Polhemus ME, Walsh DS, Yoon IK, Prosperi C, Juompan LY, Lanar DE, Krzych U, Hall BT, Ware LA, Stewart VA, Williams J, Dowler M, Nielsen RK, Hillier CJ, Giersing BK, Dubovsky F, Malkin E, Tucker K, Dubois MC, Cohen JD, Ballou WR, Heppner DG, Jr. 2010. Recombinant liver stage Antigen-1 (LSA-1) formulated with AS01 or AS02 is safe, elicits high titer antibody and induces IFN-gamma/IL-2 CD4+ T cells but does not protect against experimental *Plasmodium falciparum* infection. Vaccine 28:5135–5144. https://doi.org/10.1016/j.vaccine.2009.08.046.
141. Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, Schwenk R, Nielsen RA, Debebe Z, Pinelis E, Juompan L, Williams J, Dowler M, Stewart VA, Wirtz RA, Dubois MC, Lievens M, Cohen J, Ballou WR, Heppner DG, Jr, RTS,S Vaccine Evaluation Group. 2009. 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. https://doi.org/10.1086/600120.
142. Kester KE, Cummings JF, Ockenhouse CF, Nielsen R, Hall BT, Gordon DM, Schwenk RJ, Krzych U, Holland CA, Richmond G, Dowler MG, Williams J, Wirtz RA, Tornieporth N, Vigneron L, Delchambre M, Demoitie MA, Ballou WR, Cohen J, Heppner DG, Jr, RTS,S Malaria Vaccine Evaluation Group. 2008. Phase 2a trial of 0, 1, and 3 month and 0, 7, and 28 day immunization schedules of malaria vaccine RTS,S/AS02 in

malaria-naive adults at the Walter Reed Army Institute of Research. Vaccine 26:2191–2202. https://doi.org/10.1016/j.vaccine.2008.02.048.
143. Kester KE, McKinney DA, Tornieporth N, Ockenhouse CF, Heppner DG, Hall T, Krzych U, Delchambre M, Voss G, Dowler MG, Palensky J, Wittes J, Cohen J, Ballou WR, RTS,S Malaria Vaccine Evaluation Group. 2001. Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. J Infect Dis 183:640–647. https://doi.org/10.1086/318534.
144. Kester KE, McKinney DA, Tornieporth N, Ockenhouse CF, Heppner DG, Jr, Hall T, Wellde BT, White K, Sun P, Schwenk R, Krzych U, Delchambre M, Voss G, Dubois MC, Gasser RA, Jr, Dowler MG, O'Brien M, Wittes J, Wirtz R, Cohen J, Ballou WR, RTS,S Malaria Vaccine Evaluation Group. 2007. A phase I/IIa safety, immunogenicity, and efficacy bridging randomized study of a two-dose regimen of liquid and lyophilized formulations of the candidate malaria vaccine RTS,S/AS02A in malaria-naive adults. Vaccine 25:5359–5366. https://doi.org/10.1016/j.vaccine.2007.05.005.
145. Sedegah M, Hollingdale MR, Farooq F, Ganeshan H, Belmonte M, Kim Y, Peters B, Sette A, Huang J, McGrath S, Abot E, Limbach K, Shi M, Soisson L, Diggs C, Chuang I, Tamminga C, Epstein JE, Villasante E, Richie TL. 2014. Sterile immunity to malaria after DNA prime/adenovirus boost immunization is associated with effector memory CD8+T cells targeting AMA1 class I epitopes. PLoS One 9:e106241. https://doi.org/10.1371/journal.pone.0106241.
146. Tamminga C, Sedegah M, Regis D, Chuang I, Epstein JE, Spring M, Mendoza-Silveiras J, McGrath S, Maiolatesi S, Reyes S, Steinbeiss V, Fedders C, Smith K, House B, Ganeshan H, Lejano J, Abot E, Banania GJ, Sayo R, Farooq F, Belmonte M, Murphy J, Komisar J, Williams J, Shi M, Brambilla D, Manohar N, Richie NO, Wood C, Limbach K, Patterson NB, Bruder JT, Doolan DL, King CR, Diggs C, Soisson L, Carucci D, Levine G, Dutta S, Hollingdale MR, Ockenhouse CF, Richie TL. 2011. Adenovirus-5-vectored *P. falciparum* vaccine expressing CSP and AMA1. B. Safety, immunogenicity and protective efficacy of the CSP component. PLoS One 6:e25868.
147. Wang R, Richie TL, Baraceros MF, Rahardjo N, Gay T, Banania JG, Charoenvit Y, Epstein JE, Luke T, Freilich DA, Norman J, Hoffman SL. 2005. Boosting of DNA vaccine-elicited gamma interferon responses in humans by exposure to malaria parasites. Infect Immun 73:2863–2872. https://doi.org/10.1128/IAI.73.5.2863-2872.2005.
148. Rieckmann KH, Carson PE, Beaudoin RL, Cassells JS, Sell KW. 1974. Sporozoite induced immunity in man against an Ethiopian strain of *Plasmodium falciparum*. Trans R Soc Trop Med Hyg 68:258–259. (Letter.) https://doi.org/10.1016/0035-9203(74)90129-1.
149. Rieckmann KH, Beaudoin RL, Cassells JS, Sell KW. 1979. Use of attenuated sporozoites in the immunization of human volunteers against falciparum malaria. Bull World Health Organ 57(Suppl 1):S261–S265.
150. Stoute JA, Slaoui M, Heppner DG, Momin P, Kester KE, Desmons P, Wellde BT, Garcon N, Krzych U, Marchand M. 1997. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. RTS,S Malaria Vaccine Evaluation Group. N Engl J Med 336:86–91.
151. Clyde DF, McCarthy VC, Miller RM, Hornick RB. 1973. Specificity of protection of man immunized against sporozoite-induced falciparum malaria. Am J Med Sci 266:398–403. https://doi.org/10.1097/00000441-197312000-00001.
152. Bastiaens GJ, van Meer MP, Scholzen A, Obiero JM, Vatanshenassan M, van Grinsven T, Sim BK, Billingsley PF, James ER, Gunasekera A, Bijker EM, van Gemert GJ, van de Vegte-Bolmer M, Graumans W, Hermsen CC, de Mast Q, van der Ven AJ, Hoffman SL, Sauerwein RW. 2016. Safety, immunogenicity, and protective efficacy of intradermal immunization with aseptic, purified, cryopreserved Plasmodium falciparum sporozoites in volunteers under chloroquine prophylaxis: a randomized controlled trial. Am J Trop Med Hyg 94:663–673. https://doi.org/10.4269/ajtmh.15-0621.
153. Mira-Martinez S, van Schuppen E, Amambua-Ngwa A, Bottieau E, Affara M, Van Esbroeck M, Vlieghe E, Guetens P, Rovira-Graells N, Gomez-Perez GP, Alonso PL, D'Alessandro U, Rosanas-Urgell A, Cortes A. 2017. Expression of the *Plasmodium falciparum* clonally variant clag3 genes in human infections. J Infect Dis 215:938–945. https://doi.org/10.1093/infdis/jix053.
154. Wang CW, Hermsen CC, Sauerwein RW, Arnot DE, Theander TG, Lavstsen T. 2009. The *Plasmodium falciparum* var gene transcription strategy at the onset of blood stage infection in a human volunteer. Parasitol Int 58:478–480. https://doi.org/10.1016/j.parint.2009.07.004.
155. Lavstsen T, Magistrado P, Hermsen CC, Salanti A, Jensen AT, Sauerwein R, Hviid L, Theander TG, Staalsoe T. 2005. Expression of *Plasmodium falciparum* erythrocyte membrane protein 1 in experimentally infected humans. Malar J 4:21. https://doi.org/10.1186/1475-2875-4-21.
156. Peters J, Fowler E, Gatton M, Chen N, Saul A, Cheng Q. 2002. High diversity and rapid changeover of expressed var genes during the acute phase of *Plasmodium falciparum* infections in human volunteers. Proc Natl Acad Sci U S A 99:10689–10694. https://doi.org/10.1073/pnas.162349899.
157. de Mast Q, Groot E, Lenting PJ, de Groot PG, McCall M, Sauerwein RW, Fijnheer R, van der Ven A. 2007. Thrombocytopenia and release of activated von Willebrand Factor during early *Plasmodium falciparum* malaria. J Infect Dis 196:622–628. https://doi.org/10.1086/519844.
158. de Mast Q, van Dongen-Lases EC, Swinkels DW, Nieman AE, Roestenberg M, Druilhe P, Arens TA, Luty AJ, Hermsen CC, Sauerwein RW, van der Ven AJ. 2009. Mild increases in serum hepcidin and interleukin-6 concentrations impair iron incorporation in haemoglobin during an experimental human malaria infection. Br J Haematol 145:657–664. https://doi.org/10.1111/j.1365-2141.2009.07664.x.
159. de Mast Q, de Groot PG, van Heerde WL, Roestenberg M, van Velzen JF, Verbruggen B, Roest M, McCall M, Nieman AE, Westendorp J, Syafruddin D, Fijnheer R, van Dongen-Lases EC, Sauerwein RW, van der Ven AJ. 2010. Thrombocytopenia in early malaria is associated with GP1b shedding in absence of systemic platelet activation and consumptive coagulopathy. Br J Haematol 151:495–503. https://doi.org/10.1111/j.1365-2141.2010.08399.x.
160. De Mast Q, Sweep FC, McCall M, Geurts-Moespot A, Hermsen C, Calandra T, Netea MG, Sauerwein RW, van der Ven AJ. 2008. A decrease of plasma macrophage migration inhibitory factor concentration is associated with lower numbers of circulating lymphocytes in experimental *Plasmodium falciparum* malaria. Parasite Immunol 30:133–138. https://doi.org/10.1111/j.1365-3024.2007.01008.x.
161. Loughland JR, Minigo G, Sarovich DS, Field M, Tipping PE, Montes de Oca M, Piera KA, Amante FH, Barber BE, Grigg MJ, William T, Good MF, Doolan DL, Engwerda CR, Anstey NM, McCarthy JS, Woodberry T. 2017. Plasmacytoid dendritic cells appear inactive during sub-microscopic *Plasmodium falciparum* blood-stage infection, yet retain their ability to respond to TLR stimulation. Sci Rep 7:2596. https://doi.org/10.1038/s41598-017-02096-2.
162. Biswas S, Choudhary P, Elias SC, Miura K, Milne KH, de Cassan SC, Collins KA, Halstead FD, Bliss CM, Ewer KJ, Osier FH, Hodgson SH, Duncan CJ, O'Hara GA, Long CA, Hill AV, Draper SJ. 2014. Assessment of humoral immune responses to blood-stage malaria antigens following ChAd63-MVA immunization, controlled human malaria infection and natural exposure. PLoS One 9:e107903. https://doi.org/10.1371/journal.pone.0107903.
163. Elias SC, Choudhary P, de Cassan SC, Biswas S, Collins KA, Halstead FD, Bliss CM, Ewer KJ, Hodgson SH, Duncan CJ, Hill AV, Draper SJ. 2014. Analysis of human B-cell responses following ChAd63-MVA MSP1 and AMA1 immunization and controlled malaria infection. Immunology 141:628–644. https://doi.org/10.1111/imm.12226.
164. Teirlinck AC, Roestenberg M, Bijker EM, Hoffman SL, Sauerwein RW, Scholzen A. 2015. *Plasmodium falciparum* infection of human volunteers activates monocytes and CD16+ dendritic cells and induces upregulation of CD16 and CD1c expression. Infect Immun 83:3732–3739. https://doi.org/10.1128/IAI.00473-15.
165. Elias SC, Collins KA, Halstead FD, Choudhary P, Bliss CM, Ewer KJ, Sheehy SH, Duncan CJ, Biswas S, Hill AV, Draper SJ. 2013. Assessment of immune interference, antagonism, and diversion following human immunization with biallelic blood-stage malaria viral-vectored vaccines and controlled malaria infection. J Immunol 190:1135–1147. https://doi.org/10.4049/jimmunol.1201455.
166. Nahrendorf W, Scholzen A, Bijker EM, Teirlinck AC, Bastiaens GJ, Schats R, Hermsen CC, Visser LG, Langhorne J, Sauerwein RW. 2014. Memory B-cell and antibody responses induced by *Plasmodium falciparum* sporozoite immunization. J Infect Dis 210:1981–1990. https://doi.org/10.1093/infdis/jiu354.
167. McCall MB, Netea MG, Hermsen CC, Jansen T, Jacobs L, Golenbock D, van der Ven AJ, Sauerwein RW. 2007. *Plasmodium falciparum* infection causes proinflammatory priming of human TLR responses. J Immunol 179:162–171. https://doi.org/10.4049/jimmunol.179.1.162.
168. McCall MB, Roestenberg M, Ploemen I, Teirlinck A, Hopman J, de Mast Q, Dolo A, Doumbo OK, Luty A, van der Ven AJ, Hermsen CC, Sauerwein RW. 2010. Memory-like IFN-gamma response by NK cells following

malaria infection reveals the crucial role of T cells in NK cell activation by *P. falciparum*. Eur J Immunol 40:3472–3477. https://doi.org/10.1002/eji.201040587.
169. Rzepczyk CM, Stamatiou S, Anderson K, Stowers A, Cheng Q, Saul A, Allworth A, McCormack J, Whitby M, Olive C, Lawrence G. 1996. Experimental human *Plasmodium falciparum* infections: longitudinal analysis of lymphocyte responses with particular reference to gamma delta T cells. Scand J Immunol 43:219–227. https://doi.org/10.1046/j.1365-3083.1996.d01-24.x.
170. Teirlinck AC, McCall MB, Roestenberg M, Scholzen A, Woestenenk R, de Mast Q, van der Ven AJ, Hermsen CC, Luty AJ, Sauerwein RW. 2011. Longevity and composition of cellular immune responses following experimental *Plasmodium falciparum* malaria infection in humans. PLoS Pathog 7:e1002389. https://doi.org/10.1371/journal.ppat.1002389.
171. Walther M, Tongren JE, Andrews L, Korbel D, King E, Fletcher H, Andersen RF, Bejon P, Thompson F, Dunachie SJ, Edele F, de Souza JB, Sinden RE, Gilbert SC, Riley EM, Hill AV. 2005. Upregulation of TGF-beta, FOXP3, and CD4+CD25+ regulatory T cells correlates with more rapid parasite growth in human malaria infection. Immunity 23:287–296. https://doi.org/10.1016/j.immuni.2005.08.006.
172. Walther M, Woodruff J, Edele F, Jeffries D, Tongren J, King E, Andrews L, Bejon P, Gilbert S, Souza JD, Sinden R, Hill A, Riley E. 2006. Innate immune responses to human malaria: heterogeneous cytokine responses to blood-stage *Plasmodium falciparum* correlate with parasitological and clinical outcomes. J Immunol 177:5736–5745. https://doi.org/10.4049/jimmunol.177.8.5736.
173. Harpaz R, Edelman R, Wasserman SS, Levine MM, Davis JR, Sztein MB. 1992. Serum cytokine profiles in experimental human malaria. Relationship to protection and disease course after challenge. J Clin Invest 90:515–523.
174. Hermsen CC, Konijnenberg Y, Mulder L, Loe C, van Deuren M, van der Meer JW, van Mierlo GJ, Eling WM, Hack CE, Sauerwein RW. 2003. Circulating concentrations of soluble granzyme A and B increase during natural and experimental *Plasmodium falciparum* infections. Clin Exp Immunol 132:467–472. https://doi.org/10.1046/j.1365-2249.2003.02160.x.
175. Ockenhouse C, Hu W, Kester K, Cummings J, Stewart A, Heppner D, Jedlicka A, Scott A, Wolfe N, Vahey M, Burke D. 2006. Common and divergent immune response signaling pathways discovered in peripheral blood mononuclear cell gene expression patterns in presymptomatic and clinically apparent malaria. Infect Immun 74:5561–5573. https://doi.org/10.1128/IAI.00408-06.
176. Obiero JM, Shekalaghe S, Hermsen CC, Mpina M, Bijker EM, Roestenberg M, Teelen K, Billingsley PF, Sim BK, James ER, Daubenberger CA, Hoffman SL, Abdulla S, Sauerwein RW, Scholzen A. 2015. Impact of malaria preexposure on antiparasite cellular and humoral immune responses after controlled human malaria infection. Infect Immun 83:2185–2196. https://doi.org/10.1128/IAI.03069-14.
177. Todryk SM, Walther M, Bejon P, Hutchings C, Thompson FM, Urban BC, Porter DW, Hill AV. 2009. Multiple functions of human T cells generated by experimental malaria challenge. Eur J Immunol 39:3042–3051. https://doi.org/10.1002/eji.200939434.
178. Orlov M, Vaida F, Finney OC, Smith DM, Talley AK, Wang R, Kappe SH, Deng Q, Schooley RT, Duffy PE. 2012. *P. falciparum* enhances HIV replication in an experimental malaria challenge system. PLoS One 7:e39000. https://doi.org/10.1371/journal.pone.0039000.
179. Walker KM, Okitsu S, Porter DW, Duncan C, Amacker M, Pluschke G, Cavanagh DR, Hill AV, Todryk SM. 2015. Antibody and T-cell responses associated with experimental human malaria infection or vaccination show limited relationships. Immunology 145:71–81. https://doi.org/10.1111/imm.12428.
180. Roestenberg M, McCall M, Mollnes TE, van Deuren M, Sprong T, Klasen I, Hermsen CC, Sauerwein RW, van der Ven A. 2007. Complement activation in experimental human malaria infection. Trans R Soc Trop Med Hyg 101:643–649. https://doi.org/10.1016/j.trstmh.2007.02.023.
181. Bijker EM, Teirlinck AC, Schats R, van Gemert GJ, van de Vegte-Bolmer M, van Lieshout L, IntHout J, Hermsen CC, Scholzen A, Visser LG, Sauerwein RW. 2014. Cytotoxic markers associate with protection against malaria in human volunteers immunized with *Plasmodium falciparum* sporozoites. J Infect Dis 210:1605–1615. https://doi.org/10.1093/infdis/jiu293.
182. Dunachie S, Berthoud T, Hill AV, Fletcher HA. 2015. Transcriptional changes induced by candidate malaria vaccines and correlation with protection against malaria in a human challenge model. Vaccine 33:5321–5331. https://doi.org/10.1016/j.vaccine.2015.07.087.
183. Keating SM, Bejon P, Berthoud T, Vuola JM, Todryk S, Webster DP, Dunachie SJ, Moorthy VS, McConkey SJ, Gilbert SC, Hill AV. 2005. Durable human memory T cells quantifiable by cultured enzyme-linked immunospot assays are induced by heterologous prime boost immunization and correlate with protection against malaria. J Immunol 175:5675–5680. https://doi.org/10.4049/jimmunol.175.9.5675.
184. Tran TM, Jones MB, Ongoiba A, Bijker EM, Schats R, Venepally P, Skinner J, Doumbo S, Quinten E, Visser LG, Whalen E, Presnell S, O'Connell EM, Kayentao K, Doumbo OK, Chaussabel D, Lorenzi H, Nutman TB, Ottenhoff TH, Haks MC, Traore B, Kirkness EF, Sauerwein RW, Crompton PD. 2016. Transcriptomic evidence for modulation of host inflammatory responses during febrile *Plasmodium falciparum* malaria. Sci Rep 6:31291. https://doi.org/10.1038/srep31291.
185. Behet MC, Foquet L, van Gemert GJ, Bijker EM, Meuleman P, Leroux-Roels G, Hermsen CC, Scholzen A, Sauerwein RW. 2014. Sporozoite immunization of human volunteers under chemoprophylaxis induces functional antibodies against pre-erythrocytic stages of *Plasmodium falciparum*. Malar J 13:136. https://doi.org/10.1186/1475-2875-13-136.
186. Felgner PL, Roestenberg M, Liang L, Hung C, Jain A, Pablo J, Nakajima-Sasaki R, Molina D, Teelen K, Hermsen CC, Sauerwein R. 2013. Pre-erythrocytic antibody profiles induced by controlled human malaria infections in healthy volunteers under chloroquine prophylaxis. Sci Rep 3:3549. https://doi.org/10.1038/srep03549.
187. Woodberry T, Loughland JR, Minigo G, Burel JG, Amante FH, Piera KA, McNeil Y, Yeo TW, Good MF, Doolan DL, Engwerda CR, McCarthy JS, Anstey NM. 2017. Early immune regulatory changes in a primary controlled human *Plasmodium vivax* infection: CD1c+ myeloid dendritic cell maturation arrest, induction of the kynurenine pathway, and regulatory T cell activation. Infect Immun 85:e00986-16. https://doi.org/10.1128/IAI.00986-16.
188. Karunarathne DS, Horne-Debets JM, Huang JX, Faleiro R, Leow CY, Amante F, Watkins TS, Miles JJ, Dwyer PJ, Stacey KJ, Yarski M, Poh CM, Lee JS, Cooper MA, Renia L, Richard D, McCarthy JS, Sharpe AH, Wykes MN. 2016. Programmed death-1 ligand 2-mediated regulation of the PD-L1 to PD-1 axis is essential for establishing CD4(+) T cell immunity. Immunity 45:333–345. https://doi.org/10.1016/j.immuni.2016.07.017.
189. Loughland JR, Minigo G, Burel J, Tipping PE, Piera KA, Amante FH, Engwerda CR, Good MF, Doolan DL, Anstey NM, McCarthy JS, Woodberry T. 2016. Profoundly reduced CD1c+ myeloid dendritic cell HLA-DR and CD86 expression and increased tumor necrosis factor production in experimental human blood-stage malaria infection. Infect Immun 84:1403–1412. https://doi.org/10.1128/IAI.01522-15.
190. Mpina M, Maurice NJ, Yajima M, Slichter CK, Miller HW, Dutta M, McElrath MJ, Stuart KD, De Rosa SC, McNevin JP, Linsley PS, Abdulla S, Tanner M, Hoffman SL, Gottardo R, Daubenberger CA, Prlic M. 2017. Controlled human malaria infection leads to long-lasting changes in innate and innate-like lymphocyte populations. J Immunol 199:107–118. https://doi.org/10.4049/jimmunol.1601989.
191. Aguiar JC, Bolton J, Wanga J, Sacci JB, Iriko H, Mazeika JK, Han ET, Limbach K, Patterson NB, Sedegah M, Cruz AM, Tsuboi T, Hoffman SL, Carucci D, Hollingdale MR, Villasante ED, Richie TL. 2015. Discovery of novel *Plasmodium falciparum* pre-erythrocytic antigens for vaccine development. PLoS One 10:e0136109. https://doi.org/10.1371/journal.pone.0136109.
192. Sedegah M, Hollingdale MR, Farooq F, Ganeshan H, Belmonte M, Huang J, Abot E, Limbach K, Chuang I, Tamminga C, Epstein JE, Villasante E. 2015. Controlled human malaria infection (CHMI) differentially affects cell-mediated and antibody responses to CSP and AMA1 induced by adenovirus vaccines with and without DNA-priming. Hum Vaccin Immunother 11:2705–2715. https://doi.org/10.1080/21645515.2015.1019186.
193. Rojas-Pena ML, Vallejo A, Herrera S, Gibson G, Arevalo-Herrera M. 2015. Transcription profiling of malaria-naive and semi-immune Colombian volunteers in a *Plasmodium vivax* sporozoite challenge. PLoS Negl Trop Dis 9:e0003978. https://doi.org/10.1371/journal.pntd.0003978.
194. Kazmin D, Nakaya HI, Lee EK, Johnson MJ, van der Most R, van den Berg RA, Ballou WR, Jongert E, Wille-Reece U, Ockenhouse C, Aderem A, Zak DE, Sadoff J, Hendriks J, Wrammert J, Ahmed R, Pulendran B. 2017. Systems analysis of protective immune responses to RTS,S malaria vaccination in humans. Proc Natl Acad Sci U S A 114:2425–2430. https://doi.org/10.1073/pnas.1621489114.
195. Peng K, Goh YS, Siau A, Franetich JF, Chia WN, Ong AS, Malleret B, Wu

YY, Snounou G, Hermsen CC, Adams JH, Mazier D, Preiser PR, Sauerwein RW, Gruner AC, Renia L. 2016. Breadth of humoral response and antigenic targets of sporozoite-inhibitory antibodies associated with sterile protection induced by controlled human malaria infection. Cell Microbiol 18:1739–1750. https://doi.org/10.1111/cmi.12608.

196. Egan JE, Hoffman SL, Haynes JD, Sadoff JC, Schneider I, Grau GE, Hollingdale MR, Ballou WR, Gordon DM. 1993. Humoral immune responses in volunteers immunized with irradiated *Plasmodium falciparum* sporozoites. Am J Trop Med Hyg 49:166–173. https://doi.org/10.4269/ajtmh.1993.49.166.
197. Moreno A, Clavijo P, Edelman R, Davis J, Sztein M, Herrington D, Nardin E. 1991. Cytotoxic CD4+ T cells from a sporozoite-immunized volunteer recognize the *Plasmodium falciparum* CS protein. Int Immunol 3:997–1003. https://doi.org/10.1093/intimm/3.10.997.
198. Hollingdale MR, Appiah A, Leland P, do Rosario VE, Mazier D, Pied S, Herrington DA, Chulay JD, Ballou WR, Derks T, Yap SH, Beaudoin RL, Verhave JP. 1990. Activity of human volunteer sera to candidate *Plasmodium falciparum* circumsporozoite protein vaccines in the inhibition of sporozoite invasion assay of human hepatoma cells and hepatocytes. Trans R Soc Trop Med Hyg 84:325–329. https://doi.org/10.1016/0035-9203(90)90303-V.
199. van den Berg RA, Coccia M, Ballou WR, Kester KE, Ockenhouse CF, Vekemans J, Jongert E, Didierlaurent AM, van der Most RG. 2017. Predicting RTS,S vaccine-mediated protection from transcriptomes in a malaria-challenge clinical trial. Front Immunol 8:557. https://doi.org/10.3389/fimmu.2017.00557.
200. Chaudhury S, Ockenhouse CF, Regules JA, Dutta S, Wallqvist A, Jongert E, Waters NC, Lemiale F, Bergmann-Leitner E. 2016. The biological function of antibodies induced by the RTS,S/AS01 malaria vaccine candidate is determined by their fine specificity. Malar J 15:301. https://doi.org/10.1186/s12936-016-1348-9.
201. Schwenk R, Asher LV, Chalom I, Lanar D, Sun P, White K, Keil D, Kester KE, Stoute J, Heppner DG, Krzych U. 2003. Opsonization by antigen-specific antibodies as a mechanism of protective immunity induced by *Plasmodium falciparum* circumsporozoite protein-based vaccine. Parasite Immunol 25:17–25. https://doi.org/10.1046/j.1365-3024.2003.00495.x.
202. Sun P, Schwenk R, White K, Stoute JA, Cohen J, Ballou WR, Voss G, Kester KE, Heppner DG, Krzych U. 2003. Protective immunity induced with malaria vaccine, RTS,S, is linked to Plasmodium falciparum circumsporozoite protein-specific CD4+ and CD8+ T cells producing IFN-gamma. J Immunol 171:6961–6967. https://doi.org/10.4049/jimmunol.171.12.6961.
203. Trieu A, Kayala MA, Burk C, Molina DM, Freilich DA, Richie TL, Baldi P, Felgner PL, Doolan DL. 2011. Sterile protective immunity to malaria is associated with a panel of novel *P. falciparum* antigens. Mol Cell Proteomics 10:M111.007948. https://doi.org/10.1074/mcp.M111.007948.
204. White MT, Bejon P, Olotu A, Griffin JT, Riley EM, Kester KE, Ockenhouse CF, Ghani AC. 2013. The relationship between RTS,S vaccine-induced antibodies, CD4(+) T cell responses and protection against Plasmodium falciparum infection. PLoS One 8:e61395. https://doi.org/10.1371/journal.pone.0061395.
205. Schwenk R, Lumsden JM, Rein LE, Juompan L, Kester KE, Heppner DG, Krzych U. 2011. Immunization with the RTS,S/AS malaria vaccine induces IFN-gamma(+)CD4 T cells that recognize only discrete regions of the circumsporozoite protein and these specificities are maintained following booster immunizations and challenge. Vaccine 29:8847–8854. https://doi.org/10.1016/j.vaccine.2011.09.098.
206. Lumsden JM, Schwenk RJ, Rein LE, Moris P, Janssens M, Ofori-Anyinam O, Cohen J, Kester KE, Heppner DG, Krzych U. 2011. Protective immunity induced with the RTS,S/AS vaccine is associated with IL-2 and TNF-alpha producing effector and central memory CD4 T cells. PLoS One 6:e20775. https://doi.org/10.1371/journal.pone.0020775.
207. Vahey MT, Wang Z, Kester KE, Cummings J, Heppner DG, Jr, Nau ME, Ofori-Anyinam O, Cohen J, Coche T, Ballou WR, Ockenhouse CF. 2010. Expression of genes associated with immunoproteasome processing of major histocompatibility complex peptides is indicative of protection with adjuvanted RTS,S malaria vaccine. J Infect Dis 201:580–589. https://doi.org/10.1086/650310.
208. Sedegah M, Peters B, Hollingdale MR, Ganeshan HD, Huang J, Farooq F, Belmonte MN, Belmonte AD, Limbach KJ, Diggs C, Soisson L, Chuang I, Villasante ED. 2016. Vaccine strain-specificity of protective HLA-restricted class 1 *P. falciparum* epitopes. PLoS One 11:e0163026. https://doi.org/10.1371/journal.pone.0163026.
209. Sedegah M, Kim Y, Ganeshan H, Huang J, Belmonte M, Abot E, Banania JG, Farooq F, McGrath S, Peters B, Sette A, Soisson L, Diggs C, Doolan DL, Tamminga C, Villasante E, Hollingdale MR, Richie TL. 2013. Identification of minimal human MHC-restricted CD8+ T-cell epitopes within the *Plasmodium falciparum* circumsporozoite protein (CSP). Malar J 12:185. https://doi.org/10.1186/1475-2875-12-185.