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Controlled human malaria infection: overview and potential application in the evaluation of transmission-blocking interventions in malaria-endemic areas

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Abstract

Controlled human malaria infection (CHMI) involves the intentional infection of healthy individuals with malaria parasites, close observation of the volunteers, and clearance of the parasite at a predetermined endpoint. Depending on the need, CHMI can be initiated by either sporozoites or the administration of parasite-infected erythrocytes, with each of the two systems offering different advantages and caveats. Among other uses, CHMI has proven to be a useful tool for the evaluation of new malaria interventions, particularly vaccines and drugs. The majority of CHMI studies have been conducted in Europe, the USA and Australia, with only a handful of studies conducted in malaria-endemic countries. The slow adoption of CHMI in malaria-endemic countries may be attributed to a lack of infrastructure and expertise to conduct studies in malaria-endemic countries and the risk of undue influence and coercion as a result of volunteers' vulnerability due to a lack of education and financial situation. With the need to generate results relevant to the target populations, there has recently been an increase in CHMI studies that are being conducted in malaria-endemic countries. The use of CHMI models for the evaluation of preerythrocytic and blood-stage malaria interventions has been attempted in malaria-endemic countries with great success. There is a need for the adoption of a CHMI model for the evaluation of transmission-blocking interventions in malaria-endemic countries. The establishment of such a model in malaria-endemic countries will facilitate the selection of potential transmission-blocking intervention (TBI) candidates and accelerate their development. Here is an overview of CHMI, key challenges and ethical considerations in adopting CHMI for the evaluation of malaria transmission-blocking interventions in malaria-endemic countries.

Keywords Malaria, Controlled human malaria infection, CHMI, Transmission blocking vaccine, TBV

Background

Great progress has been made in fighting malaria worldwide; however, the malaria burden continues to be high, with an estimated 263 million malaria cases and between 597,000 and 409,000 deaths in 2023 [1]. Current malaria control efforts, such as early diagnosis and treatment, intermittent preventive treatment, and vector control, have been hampered by the spread of resistance to anti-malarial drugs [2, 3], and resistance to insecticides for mosquito control [4–6]. The development and

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application of tools that circumvent these challenges in the form of malaria vaccines would significantly increase progress toward malaria control and elimination. Malaria vaccine types include (i) preerythrocytic-stage (sporozoite and liver-stage) vaccines, (ii) blood-stage vaccines and (iii) mosquito-stage or transmission-blocking vaccines [7]. Owing to the comparatively smaller number of parasites and fewer polymorphic antigenic targets during the sexual stages of malaria infection [8, 9], transmission-blocking interventions offer attractive options that can be combined with preerythrocytic and blood-stage vaccines as a more effective approach for malaria control and elimination.

The evaluation of vaccines before licensure involves three phases (phases I, II, and III) of clinical trials and can span anywhere from approximately 10–15 years [10]. Phase II and III trials are usually large and expensive [11, 12]. Controlled human malaria infection (CHMI) involves intentionally infecting healthy individuals with malaria parasites in a controlled manner and monitoring and treating the resulting infection at a predetermined endpoint [13, 14]. CHMI studies take less time and involve fewer participants. CHMI has been widely used to evaluate different candidate malaria vaccines and drugs, providing a rapid and more cost-effective way to identify potential candidate interventions before larger trials are conducted [13, 15]. Additionally, in CHMI, fewer volunteers are subjected to experimental vaccines and procedures than in larger trials. This review provides an overview of CHMI, highlights the role of transmission-blocking interventions in malaria control and explores the significance, challenges, and ethical considerations in adapting CHMI for evaluating malaria transmission-blocking interventions in malaria-endemic regions. By providing a tool for early assessments to identify promising vaccines and drug candidates before advanced trials, CHMI could expedite the development of novel malaria interventions and result in significant cost savings.

Controlled human infection studies

In controlled human infection studies (CHISs), healthy individuals are intentionally infected with disease agents to study the disease and its treatment [16–18].

The characteristics of pathogens that can have a challenge model

CHISs are typically conducted under strict conditions that control for factors such as the type and strain of the pathogen used, the dosage and route of infection, the timing of administration, and the necessary infrastructural and safety measures required during the trial [19]. CHISs are only deemed appropriate for pathogens that cause either self-limiting diseases or can be fully managed and effectively treated [20].

History of CHIS

Human challenge studies date back to 14th May 1796, when Edward Jenner vaccinated a healthy volunteer with cowpox and later demonstrated the protection of vaccinated individuals from smallpox [16]. By the late 19th century, CHIS had become more common, with much focus on vector-borne diseases such as malaria, dengue and yellow fever. The first human malaria challenge occurred in 1898, when Battista Grassi conducted an experiment in which an individual was infected with malaria through a mosquito bite [19]. During the 1900s, deliberate malaria infections were commonly employed as a standard treatment for neurosyphilis in Europe, North and South America and India. Julius Wagner-Jauregg, an Australian psychiatrist, received the Nobel Prize in Medicine in 1927 for his discovery of this treatment approach [19, 21]. However, in the 1940s, penicillin became the recommended treatment, leading to the abolishment of this method. Currently, CHISs have various applications, including (i) the evaluation of novel vaccines and drugs and (ii) the study of the natural course of infection and immune responses to infection. These studies are cost-effective because they require smaller sample sizes and shorter durations, hence reducing the number of participants exposed to the potential risks associated with experimental interventions. [20, 22]

Infectious diseases that have challenge models

To date, many infectious diseases ranging from viral and bacterial to parasitic infections have been studied via human challenge trials. Viral infections include those caused by respiratory syncytial virus [23, 24], influenza virus [25], vaccinia Ankara [26], severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [27], norovirus [28, 29], or Norwalk virus [30]. Bacterial infections include those caused by *Shigella* spp. [31–33], *Vibrio cholerae* [34, 35], *Salmonella typhi, Salmonella paratyphi* [36, 37], *Bordetella pertussis* [38], *Neisseria meningitides* [39], *and Streptococcus pyogenes* [40], whereas tuberculosis has been studied via attenuated *Mycobacterium bovis* [41]. Parasitic infections include those caused by *Cyclospora cayetanensis* [42], *Necator americanus* [43], *Plasmodium falciparum* [44–46], *Plasmodium vivax* [47–49], and *Plasmodium malariae* [50].

Ethical issues related to CHIS

CHISs offer a method for studying different aspects of infectious diseases and evaluating and developing new interventions. Nevertheless, CHIS may raise some significant ethical dilemmas, with the obvious one being

whether intentionally infecting healthy individuals with pathogens for research purposes is morally acceptable given that there is always some risk associated with the procedure. This argument becomes valid when intentional infection with disease agents poses unacceptable levels of risk to volunteers, and it is generally expected that physicians are supposed to cure infections rather than cause them. However, meticulous planning and execution of CHIS can effectively reduce risks to participants and render CHISs ethically sound. To ensure participant safety, various measures are implemented during CHIS, including the use of low-virulence challenge strains, rigorous participant monitoring both clinically and through highly sensitive laboratory tests, and prompt diagnosis and treatment of any infections that occur [19]. For example, in Controlled Human Malaria Infections (CHMIs), parasite density is closely monitored via advanced molecular assays, and infections are terminated well before reaching levels that would typically cause clinical symptoms. Like other medical procedures, CHIS entail certain risks to participants, but since most of these risks are predictable, extensive precautions are taken to mitigate them and prevent harm.

In the context of CHIS, some groups, such as children, prisoners and incompetent adults, are considered vulnerable to exploitation. It is, therefore, recommended to avoid involving individuals from these groups in CHIS whenever possible [51]. Nonetheless, in some cases, the participation of these groups in CHIS is inevitable, especially if the intervention under study will specifically benefit that particular group [20]. The greater malaria burden in children [1] makes them the major beneficiaries of a potential malaria vaccine under study, which necessitates their inclusion in evaluation studies. Participation in a CHIS usually does not directly benefit the participants but rather exposes them to risk. However, it is ethically permissible if the risks are reasonable, full compensation for harm is provided, and participants have the ability to give voluntary informed consent to participate in the research [52]. As children cannot freely consent to participate in research, it is ethically questionable for parents to decide to enroll them in studies involving intentional harm beyond minimal risk with no direct benefit to the child [52]. Considering this, it might be safer and more ethically sound to recruit children in later studies, such as phase II or III clinical trials, when some safety data have been gathered.

It has become a general practice to financially compensate research participants, even more so when there are no direct benefits to the volunteer. Some of the reasons for this compensation include reimbursement for research-related costs such as transport and meals, compensation for time and effort, a token of gratitude, an incentive to encourage participation and sometimes a benefit to offset risks and inconveniences [53]. As noted earlier, financial compensation may result in undue influence, particularly when dealing with economically disadvantaged individuals. Although such individuals are prone to exploitation, care needs to be taken when considering exclusion from participation exclusively for financial reasons, or it may be considered discrimination [51]. To minimize the chances of undue influence, it is advised that the amount of financial compensation should be equivalent to local minimum wages for unskilled employment. The Institutional Review Board (IRB) and other reviewing authorities should evaluate and ensure that the amount of financial compensation falls within acceptable ranges. Effective community engagement and a comprehensive informed consent process empower participants to make voluntary and well-informed decisions [51, 53, 54].

Controlled human infection studies in endemic countries, experience and lessons learned

Since their inception, CHISs have been conducted primarily in developed countries, which are often equipped with experienced regulatory authorities and the technical skills necessary to oversee such high-risk research. The majority of malaria-endemic countries are low- and middle-income countries (LMICs) that may lack the technological sophistication needed to safely and effectively conduct CHIS. There are also ethical concerns about conducting CHIS in LMICs due to the lower economic status of their populations. Challenges such as obtaining informed consent and the potential for undue influence and coercion due to relatively high compensation are exacerbated in these settings because of factors such as language barriers, limited educational opportunities, and poverty [20]. However, the preference for conducting CHIS in nonendemic countries, although with good intentions, has continued to cripple the capacity of endemic countries to conduct such studies. Moreover, conducting CHIS in nonendemic countries has limitations, as the results may not apply to populations in endemic regions. Differences in immunological, genetic, and socioeconomic factors between populations from endemic and nonendemic countries may affect the generalizability of the study results. Recently, there has been a concerted effort to shift toward conducting CHIS in endemic countries while addressing the aforementioned challenges through well-defined participant selection plans and guidelines that ensure ethical considerations, including appropriate financial compensation. Consequently, several CHIS have been conducted in endemic countries, including several human malaria challenge studies in Tanzania [55–57], Kenya [58, 59], Gambia [60],

Gabon [61], and Equatorial Guinea [62], as well as cholera- and *Shigella*-related human challenge trials in Thailand [63, 64].

Early CHIS in endemic countries, particularly LMICs, encountered several challenges, including the limited infrastructure and technical capacity required to conduct and regulate such high-risk studies. During CHMI, sites relied on less sensitive thick smears for the detection of parasites, and crucial assays such as qPCR and those for measuring anti-malarial drug levels had to be performed in Europe and the USA[56, 57]. Given that local communities are often unfamiliar with the concept of CHIS, extensive community engagement is necessary to address issues related to hesitancy, trust and ethical concerns [14]. In contrast to populations from nonendemic areas, a study in Kenya reported high rates of ineligible volunteers during screening due to high degrees of previously undiagnosed comorbidities, hemoglobinopathies and asymptomatic *Plasmodium* qPCR-positive individuals [58]. To prevent unintended anti-malarial drug interference during CHMI, volunteers with recent drug use are excluded; however, laboratory tests have detected drug levels above the minimum inhibitory concentrations in some volunteers who reported no recent use. This could be attributed to the volunteers either being unaware that they had been prescribed anti-malarial drugs or having forgotten them [59]. In a follow-up study on perceptions and experiences after a CHMI trial in Kenya, some volunteers cited financial compensation as their motivation for participating in the CHMI study [14]. While this could be viewed as undue influence, it is challenging to eliminate it completely in low- and middle-income countries. When CHISs were introduced in LMICs, many countries lacked sufficient regulatory and ethical frameworks to guide the review and implementation of such studies [65].

Significant progress has been achieved in capacity building within endemic countries, largely due to strong partnerships between local research sites and international institutions. Research centers in these regions are now equipped to conduct critical procedures and tests, including the application of advanced molecular techniques for parasite detection, disease monitoring, and precise measurement of drug concentrations. These advancements have greatly enhanced the self-sufficiency of local centers and the overall quality of research. Furthermore, the availability of safety data from prior CHIS conducted in endemic areas has played a pivotal role in fostering community trust, alleviating concerns, and reducing hesitancy among potential volunteers. Challenges such as high rates of ineligibility can be effectively mitigated by screening larger pools of volunteers and strategically selecting recruitment areas tailored to the specific requirements of each study. This integrated approach ensures the continued success and ethical conduct of CHIS in endemic regions. Initiatives to develop country-specific frameworks and guidelines to oversee the review and implementation of CHIS are ongoing. Different models specific to CHIS have been discussed by Jamrozik and Selgelid [65], with guidance from the WHO available for consultation during the development of country-specific frameworks [66]. For countries with no regulatory or ethical frameworks for CHIS in place, meetings and workshops involving all stakeholders may facilitate the review process and address foreseeable issues and concerns before the submission of protocol reviews [67].

Controlled human malaria infection

Around the 1900s, deliberate malaria infections were used as a potential treatment for neurosyphilis and HIV [13, 21]. Deliberate infection with malaria later evolved into what is known today as controlled human malaria infection (CHMI), which entails intentionally infecting healthy individuals with malaria parasites in a controlled manner and monitoring and treating the resulting infection when a predetermined endpoint is reached [13]. CHMI was not used as a tool for the evaluation of malaria interventions until approximately the 1940s, when it was used in a trial to assess the efficacy of quinacrine, colchicine (SN 12,080) and quinine against Chesson strain of *P. vivax* [68]. Since then, CHMI has provided a versatile, safe and in vivo research tool for studies covering a range of areas, including the evaluation of the efficacy of malaria drugs and vaccine candidates, the study of parasite biology and virulence factors, disease control, the human immune response, parasite diagnostics, and malaria immunization strategies [13, 69]. CHMI may be initiated through a bite from an infectious mosquito, sporozoite injection, or injection of *Plasmodium*-infected red blood cells (iRBCs) (Fig. 1). The predetermined criterion for ending the infection involves reaching a specific parasite density after inoculation. CHMI studies have been performed on *P. falciparum* [44–46] and *P. vivax*. [47–49, 70], *P. falciparum*, *P. vivax* [47, 48, 71] and *P. malariae* [50], whereas *Plasmodium ovale* and *Plasmodium knowlesi* have been used only in nonhuman primate malaria infection studies [72–75].

Sporozoite-initiated controlled human malaria infection

Traditionally, sporozoite-initiated CMHI has been used for the evaluation of malaria vaccines and drugs, including those targeting the erythrocytic stages of infection [76, 77], and since sporozoite-initiated CHMI includes liver-stage infection, it is principally necessary to evaluate preerythrocytic intervention candidates [71, 78, 79]. Sporozoite-initiated infection by the bite of an infected

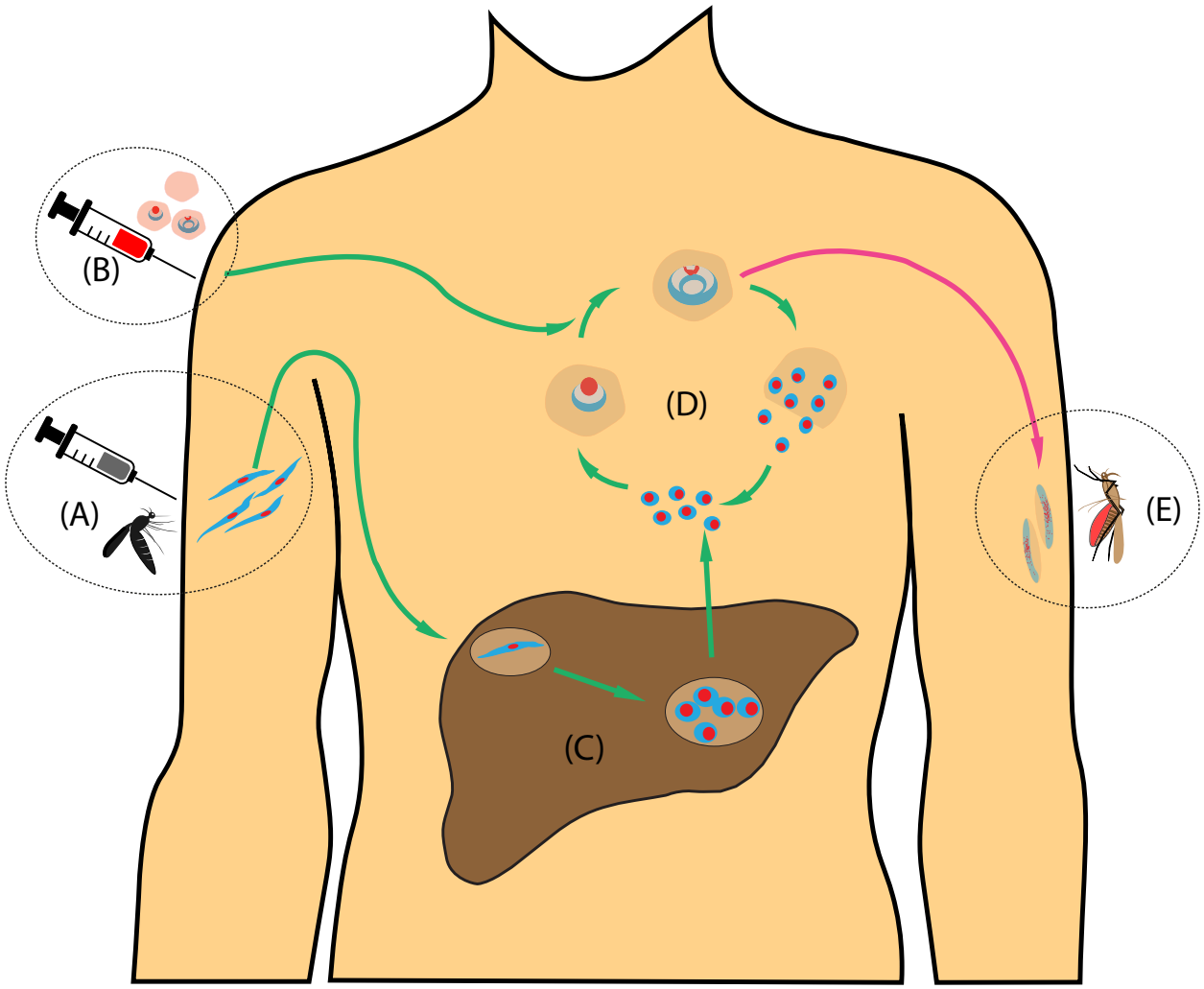

Fig. 1 Illustration of controlled human malaria infection. **A** Sporozoite initiation of malaria infection through an infective mosquito bite or the injection of a sporozoite. **B** Initiation of infection through the injection of infected red blood cells (iRBCs), which directly results in blood-stage infection and hence term blood-stage CHMI. **C** Liver stage of malaria infection. **D** Blood-stage infection. **E** Transmission of malaria parasites from humans to mosquitoes through a mosquito blood meal that contains female and male gametocytes

mosquito has the advantage of simulating the natural course of infection; however, the number of sporozoites inoculated into volunteers cannot be controlled, making it difficult to control the challenge dose [80, 81]. The delivery of cryopreserved purified sporozoites via needle injection has been used to circumvent this challenge, but again, the viable liver-to-blood inoculum (LBI) cannot be controlled, making accurate determination of the efficacy of the intervention difficult because the LBI can vary widely among participants [82].

Blood-stage controlled human malaria infection

Blood-stage CHMI is initiated through the administration of infected erythrocytes that skip the preerythrocytic stages of malaria infection [46, 83, 84]. Although both sporozoite-initiated and blood-stage CHMI models have been used to evaluate blood-stage interventions, blood-stage CHMI offers several advantages over the sporozoite-initiated model [85]. Blood-stage CHMI enables a more accurate estimation of the parasites initiating the blood-stage infection, which allows modeling of the parasite multiplication rate (PMR) with greater accuracy, hence providing greater power to detect partial efficacy of blood-stage vaccines [85, 86]. Additionally, blood-stage CHMI allows one to initiate blood-stage infection with fewer parasites than the theoretical number of merozoites released from the liver. This prolongs the period during which submicroscopic parasitaemia can be observed and studied before the development of symptoms [83]. This not only increases the number of time points for

parasitaemia data collection [84, 85] but also makes it possible to detect subtle effects of an intervention on the PMR, thus preventing premature abandonment of a partially effective vaccine that could be further optimized. As with the sporozoite-initiated infection model, the viability of the injected parasites through infected RBCs can be established only retrospectively, making it challenging to standardize the number of viable parasites initiating the infection [15]. Parasite viability has been shown to vary across different studies and sites [86], and storage conditions and the time from thawing of the parasites/parasitized RBCs to inoculation of the volunteers are the main factors for loss of viability [85]. Additionally, by circumventing the liver, the induced blood-stage challenge will not detect effects on preerythrocytic parasite stages and thus may underestimate the efficacy of vaccines that target antigens that are shared between the liver and blood stages.

Application of blood-stage controlled human malaria infection

More than 400 malaria-naïve individuals have safely participated in blood-stage CHMI, and several blood-stage malaria vaccine candidates have been evaluated via this model [87]. Very few adverse events have been reported following blood-stage CHMI than following sporozoite CHMI [88]. To address some safety concerns, *Plasmodium* cell banks are extensively tested via sensitive assays to confirm the absence of medically important bloodborne pathogens, including gram-negative and gram-positive bacteria and viruses [69, 89]. The majority of studies in naïve individuals have used a dose of up to 2800 viable iRBCs with high infection rates, but the results have not been replicated in people with NAIs. Owing to preexisting immunity, individuals with previous malaria exposure (individuals from malaria-endemic areas) have substantially lower PMRs than malaria-naïve individuals do [90], and the use of challenge doses similar to those used in malaria-naïve individuals may prolong the time to a predetermined threshold or result in self-clearance of the parasites [91]. Recently, unpublished studies in Tanzania (NCT04788862) and Mali (ISRCTN12174271) have been conducted to assess the safety and feasibility of blood-stage CHMI and the impact of varying naturally acquired immunity (NAI) on parasite growth dynamics. Through inoculum dose escalation, a study in Mali sought to identify optimal inoculum doses for the infection of individuals with differing NAI levels.

Malaria transmission-blocking vaccines

Malaria vaccines can be grouped according to the parasite stage targeted, including preerythrocytic (sporozoite and liver-stage) vaccines, blood-stage vaccines, and sexual-stage or transmission-blocking vaccines [92]. To date, two preerythrocytic malaria vaccines have been recommended by the WHO. The use of RTS and S/AS01 was recommended in October 2021, followed by the use of R21/Matrix-M in September 2023 [93–99]. Among other factors, the complexity of *P. falciparum* biology, lack of immune correlates of protection, and inadequate or short-lasting immune responses following vaccination are among the challenges facing the development of effective malaria vaccines [100].

The transmission blocking vaccines (TBVs), which aim to prevent the transmission of malaria parasites from humans to mosquitoes, are of interest in this review. These vaccines induce antibodies in the human host that target either the pre- or postfertilization stages of malaria parasites, preventing the development of transmissible forms of the parasites in the mosquito host. [92, 101]. When a mosquito ingests a blood meal from an immunized individual, it ingests these antibodies, which prevents the parasite from developing further within the mosquito, effectively blocking the transmission of malaria to another human host [9].

The potential of a transmission-blocking vaccine was demonstrated in 1958 using a bird malaria parasite, *Plasmodium gallinaceum,* where immunization of birds with *P. gallinaceum* resulted in a decreased number of oocysts in *Aedes* mosquitoes that fed on the immunized birds [101, 102]. To date, there are more than 25 published TBV antigens with confirmed transmission reducing activity (TRA) that mainly target *P. falciparum* and *P. vivax*, with a few of them targeting *Plasmodium berghei* and *Plasmodium yoelii*. [8, 92]. Currently, the leading TBV candidates in the pipeline include those based on the Pfs25, Pfs28, Pfs48/45, and Pfs230 antigens for *P. falciparum* [103–106]and the Pvs25 and Pvs28 antigens for *P. vivax* [8, 92]. The development of transmission blocking vaccines (TBVs) has faced several challenges, including (i) improper folding of the antigen protein during recombinant production, which compromises the efficacy of TBV candidates; (ii) the absence of reliable in vivo methods and models to evaluate transmission-blocking activity (TBA); (iii) the lengthy and costly nature of field trials required to establish proof of principle; (iv) inadequate stimulation of both humoral and cellular immune responses; and (v) the lack of safe combinations of adjuvants, with some antigen–adjuvant candidates being abandoned owing to safety concerns related to reactogenicity [9, 101, 107, 108].

Advantages of TBV over other types of malaria vaccines

Compared with preerythrocytic- and erythrocytic-stage vaccines, TBVs have several advantages. First, TBVs target antigens expressed during the mosquito stage of the

Plasmodium lifecycle, where sexual antigens tend to be less polymorphic owing to reduced evolutionary pressure from the immune system [9, 100]. This characteristic allows a single TBV to potentially cover more *Plasmodium* variants than vaccines targeting earlier stages of the parasite lifecycle. Second, antibodies generated from TBVs target comparatively fewer parasites in the mosquito, between 10 and 100 oocysts compared with 10^4–10^8 asexual parasites in the blood during the initial parasite multiplication cycles. This presents a significant biological bottleneck that TBVs can exploit. Additionally, TBVs are considered particularly valuable for containing outbreaks of drug-resistant parasite strains [8, 109, 110].

Although TBVs do not provide direct protection to vaccinated individuals, making them seem altruistic in nature, they have the potential to significantly reduce malaria cases and deaths across varying transmission intensities [111, 112], with their greatest impact expected in areas of low transmission [92, 113]. To address concerns about the lack of direct protection for vaccinated individuals, TBVs can be combined with vaccines targeting the preerythrocytic and erythrocytic stages of the parasite. This combination could create a multistage vaccine that not only offers individual protection but also enhances community immunity through a synergistic effect, maximizing the overall efficacy and appeal of the vaccination strategy.

Challenges in current tools for the early evaluation and prioritization of TBV

As stated earlier, early evaluation of vaccine candidates, including transmission blocking vaccines (TBVs), is crucial for prioritizing development and minimizing unnecessary costs, potentially accelerating the development of an effective malaria vaccine. Traditionally, early evaluation of TBV candidates has relied on the use of antibody tests and functional assays to assess transmission-blocking antibodies generated by TBV candidates. Enzyme-linked immunosorbent assay (ELISA) and standard membrane feeding assay (SMFA) are the most common methods for quantifying and assessing the functionality of antibodies in TBV studies. During SMFA, in vitro-produced gametocytes are mixed with either whole plasma/serum or purified immunoglobins and then fed to laboratory-reared *Anopheles* mosquitoes through a membrane feeding apparatus [100]. Transmission blocking activity (TBA) is then measured by looking at the oocyst prevalence as determined by the proportion of infected mosquitoes and infection burden as determined by oocyst density, which is usually estimated via microscopy, quantitative PCR, and immunoassays [112, 114, 115]. SMFA has been the standard method for the evaluation of TBV antibody functionality; however, the conditions during SMFA do not mimic natural malaria transmission. Different SMFA experiments have produced varying results from a single intervention [116] owing to factors such as varying gametocyte densities among assays and other interlaboratory differences. Furthermore, compared with natural infections, SMFAs involve the use of laboratory-produced gametocytes at much greater densities and do not consider other important transmission-influencing factors, such as naturally acquired immunity, which is present in natural infections. These challenges make SMFA less accurate in predicting the in vivo transmission-blocking efficacy of TBV candidates in humans.

Two mosquito feeding assays were used to assess the transmission of malaria parasites from individuals carrying gametocytes. These assays use patient blood containing gametocytes instead of cultured parasites [117, 118]. In the direct skin feeding (DSF) assay, laboratory-raised mosquitoes are placed directly on the skin, whereas in the direct membrane feeding (DMFA) assay, mosquitoes are fed whole blood samples from naturally infected individuals through a membrane. While it has been shown that there is a strong correlation in the mosquito infection rate between DMFA and DSF [119], no study has reported a good correlation between SMFA and DMFA/DSF. CHMI coupled with DSF/DMFA (Fig. 2) not only provides a potentially more accurate assessment by incorporating natural infection dynamics and host immune responses but also offers a less labor-intensive and cost-effective alternative to natural infections. DSF offers advantages, as it mimics natural infection dynamics, is more efficient than DMFA in assessing the proportion of infected mosquitoes in *P. falciparum* infections and is less susceptible to human error [119]. Furthermore, owing to sample handling errors during DMFA, temperature fluctuations can trigger gametocyte activation and gamete formation, prematurely reducing infectivity [120]. While DSF offers certain advantages over DMFA, it may present ethical and practical challenges, as some individuals may be reluctant to permit mosquito bites. Additionally, direct feeding on individuals necessitates the need to ensure that the mosquitoes used in experiments are free of potential pathogens [119].

Adaptation of blood-stage CHMI for transmission studies in malaria-endemic countries

As current malaria control strategies face challenges such as anti-malarial drug and insecticide resistance, vaccines offer promising approaches that could complement current efforts toward malaria control and eradication. There are currently approximately 20 TBV candidates at different stages of clinical development [121], and considering the challenges with traditional methods for early TBV efficacy evaluation, the

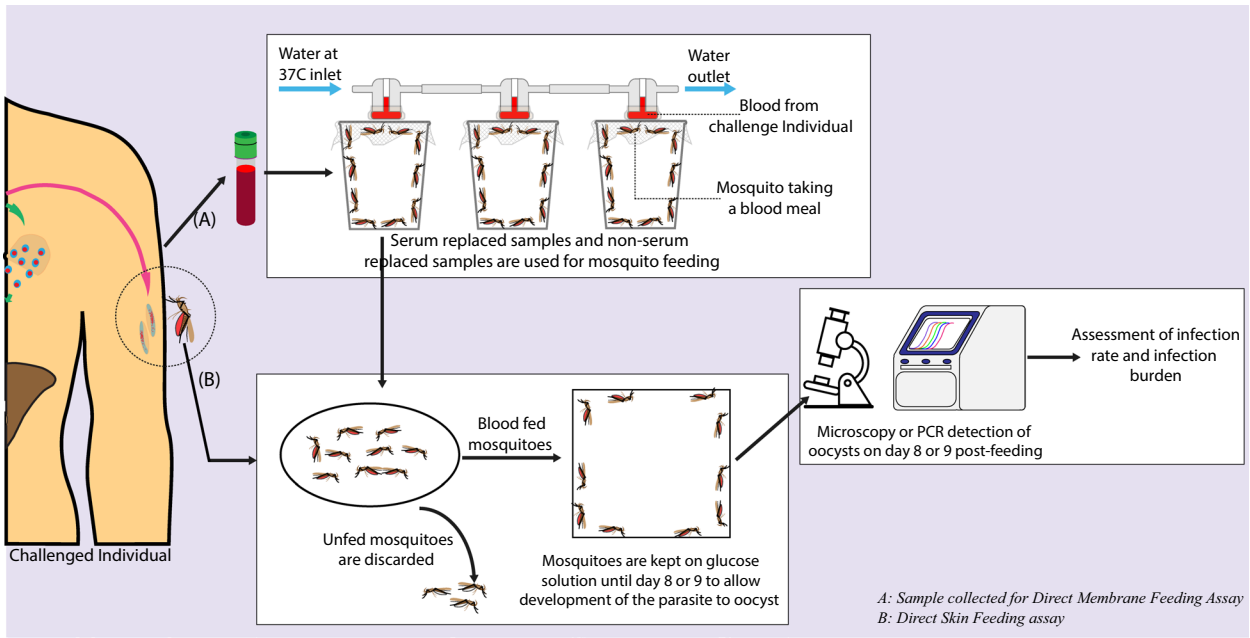

Fig. 2 Mosquito feeding assays and transmission evaluation during CHMI. Transmission evaluation during CHMI may follow. **A** Direct membrane feeding assay (DMFA), where a blood sample is collected in heparin tubes and is used to feed mosquitoes through glass feeders. **B** Direct skin feeding (DSF) assay, where mosquitoes are allowed to feed directly on challenged individuals. Both DMFA and DSF are followed by sorting mosquitoes to remove unfed mosquitoes. The blood-fed mosquitoes are then kept on glucose solution for up to 8 or 9 days when they are examined under a microscope to look for oocysts as confirmation of infection from the malaria parasite. PCR may also be used to confirm mosquito infections, and the information obtained is used to calculate infection rates and burdens

availability of an in vivo model for early evaluation of TBV would provide a more relevant tool for identifying candidates and accelerating the development of an effective TBV.

As discussed earlier, blood-stage CHMI offers distinct advantages over the sporozoite-initiated model, particularly in the assessment of transmission-blocking interventions (TBIs). The majority of the sexual antigens targeted by TBI are expressed during the erythrocytic and mosquito stages of the parasite life cycle. Hence, blood-stage CHMI streamlines the evaluation process by bypassing the unnecessary preerythrocytic stage. Additionally, blood-stage CHMI has been found to generate higher levels of gametocytes than mosquito bite-initiated CHMI does [44], thereby increasing the rate of mosquito infection during feeding assays. This highlights blood-stage CHMI as a superior option for evaluating TBI efficacy. [122]. The adaptation of the CHMI model for transmission will provide an invaluable addition to the malaria toolbox, but it is imperative that the model be established in malaria-endemic areas. Genetic and immunological differences between malaria-naïve individuals and those from malaria-endemic regions are expected to result in distinct immunological responses and parasite dynamics during CHMI. These differences may lead to variations in transmission rates and intensities between the two populations when the same transmission-blocking vaccine candidate is evaluated [123].

To implement blood-stage CHMI for transmission-blocking interventions, gametocytaemia is induced following a challenge with infected erythrocytes. This process typically involves administering subcurative doses of anti-malarial drugs with schizonticidal activity, creating stressful conditions for the parasites that encourage gametocyte formation [45, 124–126]. The subcurative doses used for gametocyte induction serve another purpose, as they also selectively attenuate the development of asexual parasitaemia, prolonging the infection to allow the development and maturation of gametocytes before the development of clinical malaria. A blood-stage CHMI model for transmission studies has been attempted in both naïve individuals [115] and in a recently conducted trial in Mali (ISRCTN12174271). In a study involving naïve individuals, gametocytes were detected in all challenged participants, and transmission to mosquitoes occurred in only 73% (7 out of 11 participants used for infections), with infection rates ranging from 2 to 17% across participant replicates [115]. Achieving higher transmission prevalence and rates would be ideal for evaluating transmission-blocking activity. Further studies to improve the model should consider ways to increase the level of gametocytemia before performing

mosquito feeding assays. Importantly, in the study by Collins, a delay in the attenuation of asexual parasitaemia with subcurative doses of piperaquine resulted in more asexual parasitaemia, gametocytaemia and eventually higher rates of transmission to mosquitoes. Although a small sample was used, the Percoll method was used to concentrate gametocytes before mosquito membrane feeding, which also increased the incidence of mosquito infection. Both approaches show potential for increasing mosquito infection prevalence and rates, thereby enhancing the overall utility of the model. This represents a significant step toward establishing a CHMI model for transmission studies, although it still leaves room for further optimization and enhancement.

Considerations for the conduct of CHMI in malaria endemic areas

As discussed earlier, previous malaria exposure can significantly affect malaria parasite growth dynamics and may result in self-clearance of the parasites [91]. Stratification of volunteers according to levels of past malaria exposure and the use of ideal doses of inoculum according to NAI levels will help to circumvent this issue. The grouping of participants according to the level of NAI has been achieved by measuring the levels of antibodies against targets such as schizonts [58, 59], merozoite surface protein 2 (MSP2) [58], membrane antigen 1 (AMA-1), merozoite surface protein 1.19 (MSP-1.19), and glutamate-rich protein (GLURP. R2) [60] by using ELISA or LumiEx assays.

Owing to the current limitation in the availability of *P. falciparum*-infected RBC banks [87], the increasing need for CHMI studies in LMICs must be met with matching efforts for capacity building to enable these countries to establish *P. falciparum*-infected erythrocyte banks. The capacity of LMICs to establish these malaria cell banks will not only improve the availability of infected RBCs but also bring them closer to research facilities addressing logistical difficulties in handling and transporting cells from Europe. Additionally, newer malaria cell banks will likely be more representative of the current field strains.

Since CHMI studies will be conducted in a malaria-endemic area, preventing the transmission of experimental malaria parasites from challenged individuals to wild mosquitoes is crucial. Some of the measures that may be taken include using mosquito repellents, applying indoor residual spraying in follow-up facilities, ensuring that participants sleep under insecticide-treated bed nets, limiting participants' interaction with the community during in-house follow-up, and providing appropriate anti-malarial treatment to clear both asexual parasites and gametocytes. Since CHMI for transmission studies involves the induction of high levels of gametocytes, ensuring that the end of the in-house follow-up treatment comprises drugs that are also effective against gametocytes is crucial. Again, measures should be implemented to prevent the escape of mosquitoes used in feeding experiments, thereby avoiding the release of the experimental parasite strain into the wild. These measures may include the use of specially designed facilities, such as insectaries housed within a secondary building, secured double-door systems, strategically placed mosquito traps, and hand-held mosquito traps [127, 128]. Laboratories working with mosquitoes/insectaries may be categorized according to the Arthropod Containment Level (ACL-1 to ACL-4) system by assessing the level of risk associated with the parasite and the vector [127].

Conclusion

CHMI studies have been pivotal in the development of malaria vaccines. As malaria researchers strive to develop next-generation vaccines with improved efficacy and broader age coverage, CHMI will remain a crucial platform for selecting the most promising candidates for further field trials. The efficacy of malaria vaccines in adults requires data generated through CHMI platforms. However, as current malaria interventions are scaled up and new interventions are introduced, demonstrating the field efficacy of new candidates in adults may become increasingly challenging. To address this challenge, advancements in the generation of multiple strains of *P. falciparum* to facilitate heterologous challenge could significantly increase the utility of CHMI platforms for novel malaria vaccine development in adults. Specifically, the adoption of blood-stage CHMI for evaluating transmission-blocking vaccines and drugs is essential for future efforts aimed at developing next-generation malaria vaccines.

Abbreviations

ACL	Arthropod Containment Level
CHMI	Controlled Human Malaria Infection
DSF	Direct Skin Feeding
DMFA	Direct Membrane Feeding Assay
ELISA	Enzyme-Linked Immunosorbent Assay
CHIS	Controlled Human Challenge Study
IRB	Institutional Review Board
iRBC	Infected Red Blood Cells
LMIC	Low and Middle Income Country
PMR	Parasite Multiplication Rate
SMFA	Standard Membrane Feeding Assay
TBI	Transmission Blocking Intervention
TBV	Transmission Blocking Vaccine

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