The Controlled Human Malaria Infection Experience at the University of Maryland

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Abstract. Controlled human malaria infection (CHMI) is a powerful tool to evaluate the efficacy of malaria vaccines and pharmacologics. Investigators at the University of Maryland, Baltimore, Center for Vaccine Development (UMB-CVD) pioneered the technique in the 1970s and continue to advance the frontiers of CHMI research. We reviewed the records of 338 malaria-naïve volunteers who underwent CHMI at UMB-CVD with *Plasmodium falciparum* from 1971 until 2017. These 338 volunteers underwent 387 CHMI events, including 60 via intradermal injection or direct venous inoculation (DVI) of purified, cryopreserved sporozoites. No volunteer suffered an unplanned hospitalization or required intravenous therapy related to CHMI. Median prepatency period was longer in challenges using NF54 (9 days) than in those using 7G8 (8 days), $P = 0.0006$ by the log-rank test. With dose optimization of DVI, the prepatent period did not differ between DVI and mosquito bite challenge (log-rank test, $P = 0.66$). Polymerase chain reaction (PCR) detected *P. falciparum* infection 3 days earlier than thick smears ($P < 0.001$), and diagnosis by ultrasensitive PCR was associated with less severe symptoms than smear-based diagnosis (39% versus 0%, $P = 0.0003$). Historical studies with NF54 showed a shorter median prepatency period of 10.3 days than more recent studies (median 11.0 days, $P = 0.02$) despite significantly lower salivary gland scores in earlier studies, $P = 0.0001$. The 47-year experience of CHMI at UMB-CVD has led to advancements in sporozoite delivery, diagnostics, and use of heterologous challenge. Additional studies on new challenge strains and genomic data to reflect regional heterogeneity will help advance the use of CHMI as supporting data for vaccine licensure.

INTRODUCTION

According to the World Health Organization (WHO), an estimated 216 million cases of malaria occurred worldwide in 2016, causing 445,000 deaths.¹ Eradication would have widespread health and economic benefits, leading to a renewed call for malaria eradication by the Bill & Melinda Gates Foundation in 2007.² Given prior failures of eradication campaigns in the 1950s and 1960s, growing resistance to artemisinins and insecticides, and increased mobility of populations, it is unlikely that malaria eradication will succeed without an effective vaccine as one tool in the armamentarium.^{3,4} Controlled human malaria infection (CHMI) offers a way to “prescreen” vaccine candidates for potential efficacy using experimental human infection in controlled conditions. Table 1 defines some of the terminology related to the discussion of CHMI. Although methodology exists for induction of blood-stage infection using parasitized erythrocytes,⁵ this review will focus on sporozoite-induced CHMI for the study of pre-erythrocytic vaccines. Prior reviews of CHMI have described the history of CHMI,^{6,7} the military experiences (Walter Reed Army Institute of Research⁶ and the Naval Medical Research Center [NMRC]),⁸ and the general clinical manifestations of CHMI.^{8,9} However, the experience at University of Maryland, Baltimore, Center for Vaccine Development (UMB-CVD) has not previously been reviewed, and we will highlight novel advances in the development of CHMI and the UMB-CVD role in this progress.

Investigators at the UMB-CVD have an extensive history of human challenge research. Dr. David Clyde pioneered the technique of using CHMI to evaluate preliminary vaccine efficacy at UMB-CVD in 1971, after using the technique as a means to evaluate new pharmaceuticals for treatment starting in 1967.^{10–12} This effort happened in parallel with a similar

effort by Dr. Karl Rieckmann with Rush-Presbyterian-St. Luke’s Medical Center working in collaboration with the NMRC.¹³ Clyde conducted these studies in an era before current strict regulatory standards without well-characterized, culture-adapted strains. Therefore, Clyde and his team used a variety of strains, acquired from patients with active malaria, from different areas of the world for immunization and challenge. “Immunization” by the bite of 379 X-irradiated *Plasmodium falciparum*-infected anopheline mosquitoes protected one of three volunteers from subsequent infection when challenged with a homologous strain from Burma.¹⁰ After additional immunization with the same strain, this volunteer remained protected on heterologous challenge with strains from Malaysia, Panama, and the Philippines as well.¹¹ One limitation of these early challenge models was the need for human volunteers to serve as the source of gametocytes for infection of the laboratory-reared anopheline mosquitoes. Because the gametocytes did not appear until the volunteers had been clinically ill for a number of days, concern for the safety and comfort of these volunteers arose during these early studies as some volunteers became notably ill. The need to time mosquito feeding on volunteers with the appearance of gametocytes in their circulation caused problems with reproducibility and efficiency of mosquito infection. The risk of transmission of potential blood-borne pathogens between volunteers by the mosquitoes also made this a less than ideal method of assessing vaccine efficacy.

Since the first CHMI studies were initiated, several advances have increased both the efficiency and scientific rigor of the method. Trager and Jensen¹⁴ outlined *in vitro* culture methods of *P. falciparum* in 1976, and Vanderberg and Gwadz¹⁵ published results showing infection of anopheline mosquitoes after feeding on these cultures through a membrane in 1980. Using these techniques, Chulay et al.¹⁶ infected six of six volunteers with the NF54 strain of *P. falciparum*, making the process of CHMI much more efficient and predictable. University of Maryland, Baltimore, Center for Vaccine Development investigators corroborated the utility of these methods in the late 1980s.¹⁷ Investigators at UMB-CVD also cloned NF54 to obtain CVD1, using it for irradiated sporozoite

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TABLE 1
Terminology associated with CHMI

Homologous CHMI	The same strain/clone of <i>Plasmodium</i> is used for vaccination and for CHMI
Heterologous CHMI	The strain/clone of <i>Plasmodium</i> used for vaccination is different from that used for CHMI
Sterile protection	Protection against patent blood-stage <i>Plasmodium</i> parasitemia during a predefined follow-up period
Delay of parasitemia onset	A delay in time to patent blood-stage <i>Plasmodium</i> parasitemia during a predefined follow-up period, compared with infectivity controls
Short-term CHMI	CHMI within 3–4 weeks after vaccination
Long-term CHMI	CHMI > 3 months after vaccination

CHMI = controlled human malaria infection.

immunization and then subsequent challenge.¹⁸ University of Maryland, Baltimore, Center for Vaccine Development researchers participated in the development of consensus documents, sponsored by the WHO in 2012, that outline the volunteer screening process, inclusion and exclusion criteria, parasite and mosquito strains, techniques for mosquito infection, primary and secondary endpoints, infectivity controls, procedures for challenge and follow-up visits, and treatment of those who develop parasitemia, as well as malaria microscopy principles and practice.¹⁹

The ethics of CHMI have evolved over time as well. Initially, researchers conducted challenge studies on prison volunteers.^{10,11,20} It is now generally considered unethical to conduct such studies in incarcerated individuals because these studies have no direct benefit for the volunteer and concerns have been raised regarding the potential for, and history of, coercion and exploitation in the conduct of medical research.²¹ For a time, CHMI trials excluded women of childbearing age because of the possibility of pregnancy. Researchers now include properly informed healthy women of childbearing age and carefully monitor adherence to parameters restricting pregnancy. Children are not currently included in CHMI trials because of the risk of severe consequences of malaria, lack of benefit to the child, and the inability of younger children to provide assent. These challenging ethical quandaries continue to limit the study of CHMI to adults, despite the fact that children and pregnant women bear the greatest burden of malaria morbidity and mortality worldwide and thus stand to benefit most from an effective vaccine,¹ which may affect the generalizability of CHMI findings.

Controlled human malaria infection has been shown to mirror vaccine efficacy in the field, making it a powerful tool for vaccine development and prioritization of resources. One of the first vaccines based on the repeat region of the circumsporozoite protein, R32ToxA, protected only one of eight volunteers challenged with CHMI.²² Field studies in Thailand and Kenya replicated this poor efficacy, showing no difference in attack rates between those vaccinated and the controls.^{23,24} Studies of RTS,S, the first licensed malaria vaccine, using differing timings, formulations, and adjuvant systems generally showed a range in efficacy of 32–57% against infection using CHMI. Field efficacy results based on time to first clinical malaria episode in Gambian and Kenyan adults showed similar percentages at 30–47% and pediatric studies showed 31–50% efficacy depending on age at first

vaccination.^{25–32} However, controversy remains in the way that CHMI and field trial results are compared. Controlled human malaria infection studies report efficacy based on the proportion of vaccine recipients who become infected with malaria as compared with the infectivity controls. The participants receive an exposure based on the number of infected mosquitoes and salivary gland density that is much more intense than that encountered in a field setting. But, strain specificity is a problem, given the limited number of culture-adapted *P. falciparum* strains. Field trials often use clinical malaria as the endpoint but differ in the definitions of clinical malaria and may use passive or active surveillance. In reporting efficacy, field trials can use time-to-event efficacy to address issues of rolling enrollment periods and loss to follow-up or Cox regression to control for covariates that may bias efficacy. Vaccine candidates continue to be routinely tested with CHMI before field studies to help define promising candidates, but further use as an endpoint for malaria vaccine licensure requires addressing these concerns.³³

Controlled human malaria infection is a critical tool in vaccine development and licensure, but notable obstacles remain. Use of mosquitoes for inoculation of sporozoites requires extensive sophisticated infrastructure including on-site insectaries. Until recently, a limited number of sites conducted CHMI studies because of cost and infrastructure requirements.¹⁹ To try to make the process easier, decrease the risk of bystander exposure to infected mosquitoes, and reduce the need for complicated infrastructure, making CHMI more accessible to other sites, including sites in endemic areas, UMB-CVD has worked with industry partners to optimize alternative routes of sporozoite delivery. Early studies required volunteers to stay on site or nearby for up to 28 days after challenge for monitoring and follow-up, making recruitment difficult. To increase sensitivity and enable earlier diagnosis, researchers at UMB-CVD developed ultrasensitive PCR (usPCR) diagnostics. Use of PCR and usPCR has enabled exclusive outpatient participant follow-up, which is now the convention at most centers that perform CHMI. In addition, earlier diagnosis and treatment reduces any inadvertent, albeit rare, in-country transmission to local mosquitoes.

Another challenge of CHMI is that the repertoire of culture-adapted *P. falciparum* strains is limited. Most CHMI challenges to date have used only the NF54 strain, a strain from a patient in the Netherlands that probably originated from West Africa, or its clone, 3D7. NF54 is one of the most commonly used vaccine strains, preventing the study of heterologous protection, an important outcome measure, given the large genetic and antigenic diversity of *P. falciparum*. Recent studies have used the 7G8 clone of Brazilian origin, and newer strains are under consideration, including NF135.C10 and NF166. NF135.C10 is of Cambodian origin and is resistant to chloroquine but susceptible to other commonly used antimalarials.³⁴ NF166 originates from Guinea and is susceptible to all tested commonly used antimalarials.³⁵ At this time, only these four culture-adapted strains are available for CHMI, a potential limitation for generalizability of results to malaria-endemic areas. For instance, none of these strains is from East Africa, a region with large parasite diversity. Several vaccine studies at UMB-CVD have used heterologous challenge to further study the strain specificity of the vaccine. This report summarizes our experiences with CHMI over the last 47 years, focusing on how the advancements mentioned in this paragraph

have changed CHMI trials at UMB-CVD and how these advancements can help inform the future of CHMI overall.

MATERIALS AND METHODS

Controlled human malaria infection procedures. Relying on the expertise of the limited number of centers that conduct CHMI worldwide, including UMB-CVD, the WHO sponsored the development of a consensus document titled “Standardization of Design and Conduct of *P. falciparum* Sporozoite Challenge Trials” that provides guidelines for conduct of CHMI.¹⁹ All CHMI trials are conducted in compliance with the Declaration of Helsinki with appropriate informed consent and approval by the institutional review board (IRB) of the University of Maryland and any other relevant IRBs. We carefully screen volunteers to mitigate noncompliance and seek the participant’s permission to designate two contact individuals who are aware of the participant’s participation in the study whom we can contact in the event that we cannot reach the participant directly. We have standard operating procedures in place in the unlikely event of a missing volunteer and institutional backup at the level of the Dean’s Office to institute safeguards in the event that we need to trace volunteers to ensure their personal safety. We have had extensive safety discussions with the Food and Drug Administration (FDA) and maintain a designated challenge center under master file.

Controlled human malaria infection is extremely safe with more than 1,500 volunteers having undergone CHMI without any deaths reported.^{6,8,9} One center has reported two separate cardiac cases after participation in CHMI studies. One of

the participants had a moderate risk of coronary event within 10 years on later review, so trials at UMB-CVD now exclude all volunteers with more than a 10% chance of a coronary event in the 5 years after the planned study, as well as anyone with significant abnormalities on screening electrocardiograms.

To ensure homogeneity of responses to vaccines and CHMI, only malaria-naïve participants are included in the trials. Anyone with any known history of malaria infection, anyone who has been a long-term resident (> 5 years) of a malaria-endemic area, anyone who was born and resided in a malaria-endemic area, or anyone who has traveled to a malaria-endemic area within the previous 6 months is excluded.

Initial studies carried out at UMB-CVD by Dr. David Clyde testing immunization with irradiated, infected mosquitoes used gametocyte donors and a paired (split feed) technique for exposure to infective mosquitoes. In this technique, half of the mosquitoes fed first on the immunized subject and then were moved to the control subject to finish their feeding, whereas the other half of the mosquitoes were placed in the reverse order.¹⁰ The infectivity controls ensured that the mosquitoes were infectious at the time of the feeding and that a lack of infection of the immunized subject did not represent a lack of exposure to malaria. Advances in culture techniques and membrane feeding have allowed for reliable production of gametocytes, so gametocyte donors are no longer necessary. University of Maryland, Baltimore, Center for Vaccine Development began using these techniques in malaria challenges beginning in the mid-1980s.^{17,36} In the modern mosquito bite model (Figure 1), challenges use *Anopheles stephensi* mosquitoes infected with NF54, 3D7 (a clone of NF54), or

FIGURE 1. Schematic of controlled human malaria infection. **(A)** The volunteer is infected with sporozoites either through the bite of an infectious mosquito or direct venous inoculation with cryopreserved infectious sporozoites (1). The sporozoites travel to the liver (2), where they enter hepatocytes and mature into schizonts (3). These schizonts rupture and release merozoites into the bloodstream (4). Merozoites enter erythrocytes and become ring stage trophozoites (5), mature into schizonts and rupture releasing more merozoites into the bloodstream to continue the cyclic rise in parasitemia (6) (gametocyte stages not shown). **(B)** Cyclic rise in parasitemia. Merozoites exit the liver approximately 7 days after infection. Asexual reproduction in erythrocytes results in a cyclic rise in parasitemia. Parasitemia becomes detectable at around 16 parasites/mL for ultrasensitive PCR (a), 40 parasites/mL for conventional PCR (b), and about 2,000 parasites/mL (2 parasites/ μ L) (c) for thick blood smear.

7G8 strains of *P. falciparum*. Mosquitoes are reared and *P. falciparum* strains are cultured as previously detailed, with small variations in some protocols.³⁷ Five female mosquitoes are allowed to feed on a volunteer's arm. The mosquitoes are then dissected to confirm that they have taken a blood meal and to score the density of salivary gland infection. By convention, the number of sporozoites present in mosquito salivary glands is categorized as 0 (no sporozoites), 1 (1–10), 2 (11–100), 3 (101–1,000), and 4 (> 1,000).³⁸ If required, additional mosquitoes are allowed to feed until five mosquitoes with a minimum salivary gland score of two feed on the volunteer. Blood-stage parasites are typically visualized by thick blood smear between 10 and 11 days after challenge (range 7–18 days) and are detected by PCR between 8 and 9 days after challenge (range 7–16 days). Earlier diagnosis has permitted us to move to an outpatient monitoring model in which participants are asked to return for daily visits starting on or around 5 days after challenge for history, vital signs, physical examination, and malaria diagnostics. To be considered positive, a study participant must have two positive PCRs within 60 hours of each other to account for cyclic waxing and waning around the lower level of detection. Once parasitemia is confirmed by two positive PCRs, directly observed antimalarial therapy is given over 48 hours on an outpatient basis. Appropriate antimalarial therapy is dependent on the drug resistance status of the challenge strain. The NF54 strain of *P. falciparum* malaria and its clone, 3D7, are sensitive to chloroquine, but the 7G8 strain is resistant and requires artemisinin combination therapy or atovaquone–proguanil.

Researchers at UMB-CVD have also studied alternative routes of delivery of sporozoites. In a study on intradermal (ID) injection, 30 malaria-naïve adults aged 18–45 years received ID injection of cryopreserved *P. falciparum* sporozoites of the NF54 strain using a 25-gauge needle and syringe with six different groups receiving different doses, aliquot volumes, and numbers of injections.³⁹ Those who did not develop malaria by day 28 after CHMI received treatment with atovaquone–proguanil to ensure clearing of potentially delayed infections.³⁹ Another study carried out at UMB-CVD aimed to establish the proper dosing for effective transmission of 7G8 strain of *P. falciparum* by direct venous inoculation (DVI) of sporozoites. A total of 30 participants underwent CHMI by direct venous injection of the 7G8 strain at a variety of doses or the NF54 strain at what was considered a standard dose (NCT02780154). These participants underwent outpatient follow-up as detailed in the last paragraph and received treatment with atovaquone–proguanil if they did not develop malaria by 28 days after challenge.

Malaria diagnostics. The gold standard for malaria detection worldwide is thick blood smear. In studies at UMB-CVD in which this is used for diagnosis, a 10- μ L aliquot of blood is placed on a microscope slide in a 1 $\times$ 2 cm rectangle with Giemsa staining for the creation of a thick smear beginning around 5 days after challenge. Investigators examine five separate passes along the 1-cm axis of the blood smear using the $\times$ 100 oil immersion lens of calibrated microscopes. This approximates 0.1 μ L of blood per pass. Parasites can be quantified with a sensitivity of 2 parasites/ μ L by this technique (Figure 1B). A positive smear is defined as having at least two unquestonable *P. falciparum* parasites confirmed by at least one investigator and the expert reader. For alternative diagnostics, real-time PCR with or without quantification has been

optimized at UMB-CVD, which has a sensitivity of $\sim$ 40 parasites/mL (Figure 1B).³⁷ At this point, most challenge studies have progressed from dual methodology for the diagnosis of malaria and documentation of clearance after treatment (with the blood smear being the definitive diagnosis) to using PCR exclusively for diagnosis and documentation of clearance by day 28.^{40,41} More recent studies at UMB-CVD have used novel ultrasensitive reverse transcription quantitative PCR (usRT-PCR) techniques, which has a sensitivity of $\sim$ 16 parasites/mL (Figure 1B).⁴² PCR primers for usPCR amplified the *Plasmodium* 18S ribosomal RNA genes, as well as the ribosomal RNA gene transcripts.^{42,43} Daily results were analyzed data with the Roche LightCycler® 96 software version 1.1 or the LightCycler®480 software 1.5.1.

Record review. We reviewed the records of 338 unique volunteers who underwent 387 separate episodes of CHMI with *P. falciparum* at the UMB-CVD between 1971 and 2017 (Table 2). Only those patients who underwent CHMI with culture-adapted strains were included in the final analysis.

Statistics. New analyses of compiled data used GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA).

RESULTS

Details regarding sample sizes, number of participants vaccinated, number of challenges performed, and mean prepatent period for each study are presented in Table 2. With dose optimization, the number of days non-vaccinated participants remained malaria free by PCR was not significantly different between participants who received sporozoites via DVI compared with those who received mosquito bite challenge with five qualifying bites of NF54 (log-rank test, $P = 0.66$, Figure 2A). Participants who underwent challenge by ID injection had a significantly longer median time to positive PCR, 11 days, as compared with mosquito bite challenge and DVI challenge with either strain, 9 days for all other groups (log-rank test, $P = 0.028$, Figure 2A).

The median number of days to patent infection was a full day longer for those undergoing mosquito bite challenge with five qualifying bites of NF54 (9 days) than for those undergoing mosquito bite challenge with five qualifying bites of 7G8 (8 days), $P = 0.0006$ by log-rank test (Figure 2B). This may be confounded by PCR methodology, as trials that included a 7G8 challenge generally used us-PCR, whereas earlier trials that included an NF54 challenge used standard PCR. However, clinical experience at our site leads authors to believe that there are inherent differences between the strains, as salivary gland counts tend to be higher in mosquitoes infected with 7G8 and there have, thus far, not been instances of uninfected infectivity controls challenged with 7G8 by mosquito bite.

Interestingly, the prepatency period by thick smear was shorter in CHMI trials performed in the 1980s–1990s using NF54 (median prepatent period 10.3 days) than in more recent studies using NF54 (median prepatent period 11.0 days, $P = 0.02$, log-rank test, Figure 3). Geometric mean prepatent periods were 9.6 days for trials performed in the 1980s–1990s and 11.2 days for more recent studies. This analysis includes only non-vaccinated participants and only those who underwent mosquito bite challenge with five qualifying bites. Although data could be obtained for only 14 of the 20 participants for earlier studies, geometric mean salivary gland scores were

FIGURE 2. **(A)** Prepatent period by PCR stratified by route of administration. Depicted is the prepatent period (time to positive *Plasmodium falciparum* [Pf] PCR) for non-vaccinated participants who received *P. falciparum* sporozoites via mosquito bite, direct venous inoculation (DVI), or intradermal (ID) administration and stratified by *P. falciparum* strain. No significant difference was observed between mosquito bite administered sporozoites and DVI administered sporozoites of either strain (median time to positive PCR of 9 days for all, log-rank test, $P = 0.66$). Challenge by ID injection resulted in a significantly longer median time to positive PCR, 11 days, compared with DVI challenge with either strain and mosquito bite challenge, 9 days for all other groups (log-rank test, $P = 0.028$). Geometric mean prepatent period was 10.6 days for challenge by ID injection, 9.4 days for NF54 challenge by DVI, 9.3 days for 7G8 challenge by DVI, and 8.8 days for NF54 challenge by mosquito bite. Prepatent period was counted in whole days with half days being possible if the participant had two follow-ups in 1 day. Mosquito bite challenge includes only participants who received five qualifying bites of NF54 challenge, DVI with 7G8 challenge includes only participants who received > 3,200 sporozoites of 7G8 via DVI, DVI with NF54 (Sanaria™ *P. falciparum* sporozoites [PfSPZ] Challenge) includes only participants who received 3,200 sporozoites of Sanaria PfSPZ Challenge via DVI, and ID includes participants who received 10,000–50,000 sporozoites of NF54 in two or eight injections. A bite qualifies in mosquito bite challenges if the mosquito had evidence of a blood meal and a sporozoite gland count of > 10 ($\geq 2+$). **(B)** Prepatent period by PCR stratified by *Pf* strain. Depicted is the prepatent period as detected by real-time PCR and stratified between *Pf* NF54 and *Pf* 7G8 clone used in challenge. The median number of days to positive *Pf* PCR was a full-day longer for NF54 (9 days) as compared with 7G8 (8 days), and the time-to-event curves were significantly different by log-rank test, $P = 0.0006$. Geometric mean prepatent period for mosquito bite challenges with NF54 was 8.8 days and for 7G8 was 7.8 days. Prepatent period was counted in whole days with half days being possible if the participant had two follow-ups in 1 day. Data include only mosquito bite challenge with five qualifying bites. A bite qualifies in mosquito bite challenges if the mosquito had evidence of a blood meal and a sporozoite gland count of > 10 ($\geq 2+$). This figure appears in color at www.ajtmh.org.

significantly lower for trials performed in the 1980s–1990s compared with newer studies, 2.7 versus 3.5, $P = 0.0001$ by unpaired *t*-test.

In the entire experience of UMB-CVD, no related unplanned hospitalizations have occurred and no invasive interventions, such as IV antimalarial therapy or rehydration or unplanned diagnostic tests, have been required. This is in line with other centers' experiences, showing that CHMI is extremely safe with proper procedures and risk mitigation.^{6,8} As stated before, CHMI studies at UMB-CVD now usually use an outpatient monitoring model, with 247 volunteers followed safely as outpatients so far. These studies used traditional PCR or usPCR as the diagnostic assay for malaria. PCR detected prepatent infection a mean of 3 days earlier than thick smears (range 0–6 days earlier, Table 3, calculated by two-way analysis of variance (ANOVA) with Bonferroni posttests), consistent with previously published data.⁵⁰ When comparing an earlier study using smear diagnosis³⁷ with more recent

studies using usPCR (unpublished data), no patients in the group diagnosed by usPCR had severe symptoms, compared with 14 (39%) in the group diagnosed by thick smear, all of these being fever higher than 102.2°F ($P = 0.0003$ by Fisher's exact test). Fewer participants in trials using usPCR for diagnosis had mild to moderate headache ($P < 0.01$), malaise ($P < 0.05$), and chills ($P < 0.01$) associated with the malaria event (Table 4, calculated by two-way ANOVA with Bonferroni posttests). Of note, usPCR symptoms contain data for sporozoites delivered by DVI and mosquito bite, and both PCR and usPCR symptoms include data from trials using NF54 and 7G8 challenge strains/clones, which may introduce confounding. However, when comparing symptoms associated with sporozoite delivery via mosquito bite and sporozoite delivery via DVI both using usPCR as the primary diagnostic, no significant differences between symptoms were found. Earlier diagnosis, combined with less symptoms associated with malaria, has allowed us to move to an outpatient model,

FIGURE 3. Prepatent period by thick blood smear for challenges before 1991 and those since 2008. Historical studies with NF54 showed a shorter median prepatent period, 10.3 days, as compared with more recent studies, 11.0 days (log-rank test, $P = 0.01$). Geometric mean prepatent period in historical studies was 9.6 days and in more recent studies was 11.2 days. Prepatent period was counted in whole days with half days being possible if the participant had two follow-ups in 1 day. Geometric mean sporozoite gland count for challenges before 1991 was 2.7 compared with 3.5 in newer studies, $P = 0.0001$ by unpaired t -test.

dependent on study aims and outcome, improving volunteer compliance, increasing recruitment numbers and lowering overall study cost.

DISCUSSION

University of Maryland, Baltimore, Center for Vaccine Development has been at the forefront of innovations in the field of CHMI. In addition to being the first institution to conduct CHMI to test the efficacy of a possible vaccine,¹⁰ researchers at UMB-CVD have led advances in the field in terms of CHMI route, strains, diagnostics, and safety. Studies carried out at UMB-CVD have helped to optimize both mosquito-based and parenteral challenges,^{37,39,48} with studies of DVI using NF54 and 7G8 recently completed (manuscript in preparation). The findings that days to patency were not significantly different between mosquito bite challenge and DVI with NF54 and 7G8 are encouraging as we look toward the future of CHMI. Direct venous inoculation requires less infrastructure, such as insectaries and complex challenge suites, making the possibility of expanding CHMI to other sites, including field sites, more feasible. One recently completed trial of a radiation-attenuated *P. falciparum* sporozoite vaccine used DVI for CHMI in Tanzania⁵¹ and several clinical trials that will use CHMI via DVI in endemic countries are currently recruiting (NCT03420053 and NCT02739763). Controlled human malaria infection in endemic populations allows researchers to study the effects of preexisting immunity on the efficacy of vaccines and drugs

more directly and more efficiently than natural infection. With the expansion of the strains validated for DVI, researchers can also examine heterologous protection of vaccines in semi-immune populations in a more direct and controlled manner.

Researchers at UMB-CVD have also developed procedures for the use of PCR, including usPCR, for the detection of parasitemia in volunteers undergoing CHMI, and have participated in external quality assurance to ensure validation of these assays.^{37,52} As expected, the use of PCR in CHMI trials results in earlier diagnosis than thick smear (Table 3). Using PCR, and particularly usPCR, results in less symptoms associated with the malaria event (Table 4). These features enable the safe conduct of CHMI in the outpatient setting, which places less demands on volunteers, thus improving recruitment and retention.

Heterologous challenge studies at UMB-CVD have evaluated strain-transcending protection with the 3D7 and the 7G8 strains.⁴⁰ Mosquito bite challenge with the 7G8 parasite resulted in a 1-day shorter prepatent period than mosquito bite challenge with NF54 and call into question whether inherent differences between the strains exist. Further head-to-head comparisons of culture-adapted strains for CHMI should be considered as the number of strains continues to increase.

The significant difference in median prepatent period by thick smear between studies carried out in the 1980s–1990s compared with more recent studies raise concern that culture-adapted NF54 may be attenuating over time. Geometric mean salivary gland scores were significantly higher for the newer studies. Procedures for diagnosis of malaria in volunteers have changed only slightly. Thick blood smears were obtained more frequently (every 12 hours) in studies from the 1980s to 1990s compared with more recent studies (daily but more frequently if symptomatic), but this would only account for a half-day difference in prepatent periods seen. There was a 0.7-day difference in median prepatent period and a 1.6-day difference in geometric mean prepatent period between the two time periods. Procedures for examining thick blood smears are more thorough in more recent studies, with at least 250 oil immersion fields examined in recent studies compared with at least 200 oil immersion fields in older studies. Therefore, we believe that the difference cannot be explained by differences in methodology alone. Adding additional culture-adapted strains to the CHMI repertoire and doing head-to-head comparisons between the strains could help better define the characteristics of CHMI associated with each strain and guide the use of these strains in future drug and vaccine trials.

As the burden of malaria declines at established field sites, CHMI becomes an even more important tool to mitigate the costs associated with large-scale Phase 2b and 3 trials to

TABLE 3
Difference in prepatent period in unvaccinated participants, PCR compared with thick smear*

Study	Mean prepatent period by PCR, days	Mean prepatent period by thick smear, days	Difference in prepatent period, days	95% confidence interval	Significance testing
NF54 challenge (n = 36) (aseptic mosquitoes)	7.5	10.9	3.4	2.8–3.9	$P < 0.001$
NF54 challenge (n = 18) (non-aseptic mosquitoes)†	8.7	11.5	2.8	1.8–3.8	$P < 0.001$
Parenteral challenge (n = 24) (intradermal)	10.7	13.6	2.9	2.0–3.7	$P < 0.001$

* Polymerase chain reaction detected *Plasmodium falciparum* infection 3 days earlier than thick smears (two-way ANOVA with Bonferroni posttests), as has been shown in other studies.⁵¹ Our center has not performed head-to-head trials comparing prepatent period by blood smear and PCR for 7G8 trials.

† Calculated using two-way ANOVA with Bonferroni posttests.

‡ This PCR was performed at the National Institutes of Health with a different methodology and different sensitivity from those used in the other two trials.

TABLE 4
Symptoms associated with malaria event, thick blood smear compared with PCR and usPCR

Symptoms	Thick smear (n = 36)	PCR (n = 30)	usPCR (n = 24)	Thick smear (n = 36)		PCR (n = 30)		usPCR (n = 24)	
	N (%)	N (%)	N (%)	Mild to moderate N (%)	Severe N (%)	Mild to moderate N (%)	Severe N (%)	Mild to moderate N (%)	Severe N (%)
Fever	29**†† (80.6)	1 (3.3)	2 (8.33)	15 (41.7)	14*† (38.9)	1 (3.3)	0 (0)	2 (8.3)	0 (0)
Headache	29 (80.6)	15 (50.0)	7 (29.2)	30 (83.3)††	0 (0)	15†† (50.0)	0 (0)	7 (29.2)	0 (0)
Malaise	26 (72.2)	9 (30.0)	6 (25.0)	26 (72.2)†	0 (0)	9† (30.0)	0 (0)	6 (25.0)	0 (0)
Chills	27† (75.0)	8 (26.7)	4 (16.7)	27 (75.0)*††	0 (0)	8†† (26.7)	0 (0)	4 (16.7)	0 (0)
Myalgia	26 (72.2)	11 (36.7)	8 (33.3)	26 (72.2)	0 (0)	11 (36.7)	0 (0)	8 (33.3)	0 (0)
Nausea	16 (44.4)	7 (23.3)	6 (25.0)	17 (47.2)	0 (0)	7 (23.3)	0 (0)	6 (25.0)	0 (0)
Dizziness	13 (36.1)	6 (20.0)	1 (4.2)	13 (36.1)	0 (0)	6 (20.0)	0 (0)	1 (4.2)	0 (0)
Arthralgia	16 (44.4)	5 (16.7)	4 (16.7)	16 (44.4)	0 (0)	5 (16.7)	0 (0)	4 (16.7)	0 (0)
Diarrhea	5 (13.9)	2 (6.7)	3 (12.5)	5 (13.9)	0 (0)	2 (6.7)	0 (0)	3 (12.5)	0 (0)

usPCR = ultrasensitive PCR. Participants in trials using thick smear for diagnosis had significantly more fever than those in trials using either PCR or usPCR for diagnosis ($P < 0.01$). In trials using usPCR for diagnosis, participants had significantly less mild to moderate headache, malaise, and chills than participants in trials using thick smear for diagnosis and those using PCR for diagnosis. Only participants in trials using thick smear for diagnosis had severe symptoms, with all of the severe symptoms being fever $> 102.2^{\circ}\text{F}$.

* Significant difference between thick smear and PCR, two-way ANOVA with Bonferroni posttests ($* = P < 0.05$, $** = P < 0.01$).

† Significant difference between thick smear and usPCR, two-way ANOVA with Bonferroni posttests ($\dagger = P < 0.05$, $\dagger\dagger = P < 0.01$).

‡ Significant difference between PCR and usPCR, two-way ANOVA with Bonferroni posttests ($\ddagger = P < 0.05$, $\ddagger\ddagger = P < 0.01$).

study vaccine efficacy. Given the correlation of CHMI with field efficacy in the study of R32ToxA^{22–24} and RTS,S,^{25–32} researchers have started to discuss how CHMI data may play a role in vaccine licensure.^{33,53} Using CHMI to demonstrate efficacy for vaccine licensure facilitates both cost- and time-efficient vaccine development, bringing effective vaccines to market more rapidly than the traditional field study model. This may be of particular importance for a traveler's vaccine. The successful vaccine licensure by the FDA of the PaxVax CVD 103-HgR (Vaxchora[®]) vaccine after a trial at UMB-CVD evaluating the efficacy against human experimental infection with *Vibrio cholera* O1 El Tor presents a model of human experimental infection as a pathway to licensure.^{54,55} However, licensure of a malaria vaccine will require a combination of CHMI and field trials and will not be as straightforward as cholera, given the heterogeneity of malaria in endemic countries. It is also not clear as to how many challenge strains would adequately represent the diversity of epitopes seen immunologically after exposure in malaria-endemic regions. Ongoing genomic and transcriptomic studies of malaria strains may enable a more strategic approach toward strain utilization for CHMI and vaccine development. We envision CHMI as a powerful tool to not only bring vaccines to field-testing but as a potential pathway to licensure as well. Controlled human malaria infection can also play a role in post-licensure research to examine the effects of different doses and schedules of administration of vaccines to improve efficacy.

Future directions for CHMI will need to include a larger variety of strains coupled with genomic data to reflect regional heterogeneity as the most efficient means of determining vaccine efficacy. Follow-up studies carried out on the Phase 3 clinical trial of RTS,S/AS01 in African children aged 5–17 months showed that there was higher efficacy of the vaccine at 1 year for parasite strains whose sequence matched the vaccine strain.⁵⁶ If a larger variety of strains is available for heterologous challenge, trials could test new vaccines against several strains to determine whether they will have similar differential field efficacy or if they appear to provide strain-transcending protection. In addition, given our results demonstrating a significant difference in the mean prepatent period by thick smear comparing studies from the 1980s to 1990s with more recent studies, concerns about attenuation of culture-adapted NF54 over time may be warranted. A diversification of culture-adapted strains will help mitigate some of this concern.

CONCLUSION

University of Maryland, Baltimore, Center for Vaccine Development's 47-year experience of using CHMI as a barometer of vaccine efficacy has enabled resource allocation to advance the most promising vaccine candidates to more expensive Phase 2b trials. Indeed, it is now considered standard to test the efficacy of a new candidate malaria vaccine with CHMI before pursuing field trials. We hope that these models can serve as guidance for future vaccine development, not only in the field of malaria but also in other diseases such as dengue that are logistically challenging and expensive to study in the field, yet are of great public health importance.

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