Blocking the cycle

Antimalarial and immunisation strategies to target *Plasmodium falciparum* transmission

Merel Janneke Smit

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CHAPTER 1

General Introduction

The historical burden of malaria

As of October 2024, Egypt was officially declared malaria-free by the World Health Organization (WHO),[1] marking a historic achievement in the global fight against the disease. Malaria had been endemic in Egypt for millennia, with evidence tracing it as far back as 4000 B.C.E. Ancient Egyptian mummified remains, including that of the 18th Dynasty pharaoh Tutankhamun, have revealed traces of the *Plasmodium* parasite,[2] suggesting malaria may have played a role in his untimely death. While the exact cause of death remains debated, the discovery of the parasite in his remains underscores the long-standing presence of the disease.

Throughout human history, malaria has been among the most lethal infectious diseases. When the entire human history is considered, malaria has claimed more lives than any other disease. Malaria was widespread in many areas, from Neolithic dwellers to early Chinese and Greeks civilisations. In Europe, malaria was endemic for centuries and continued to pose a health burden well into the 20th century. During the time of the Roman Empire, the Pontine Marshes near Rome were infamous for malaria outbreaks.[3] Historical reconstructions suggest that up to 50% of all deaths in parts of central and southern Italy during the imperial period may have been malaria-related.[3] The term "malaria" itself derives from the Italian words mal'aria, meaning "bad air".[4] This name reflects the ancient misconception that the disease was caused by foul air from swamps and marshes, where mosquitoes thrived.

In many endemic regions, malaria was a major cause of mortality and economic hardship. Historical records indicate that in East Africa during the early 20th century, up to 50% of native children died before the age of four, predominately due to malaria.[5] The disease was also a constant threat to European colonists—whether merchants, missionaries, military personnel, or migrants—hindering expeditions of discovery and commerce in the tropics. In West Africa, malaria was so deadly to European newcomers that it earned the nickname "the White Man's Grave". Mortality rates among European colonists were extremely high—50% to 70% died within a year of arrival.[6] This reality was captured in the refrain of a 19th century British sea shanty: *"Beware and take care of the Bight of Benin. There's one that comes out for forty goes in."*.[7]

During World War II, malaria posed such a threat to military personnel that, in certain regions of the war, it is estimated that malaria incapacitated more

troops than all other causes combined, including battle wounds and injuries.[8] This was particularly true in the Pacific and Southeast Asia,[9] where malaria was endemic and the tropical environment provided ideal breeding conditions for the *Anopheles* mosquitoes. The dense jungles, heavy rainfall, and high humidity made it nearly impossible to avoid mosquito bites, contributing to the widespread transmission of the disease.

It wasn't until the late 19th century that scientists identified Plasmodium parasites, transmitted by *Anopheles* mosquitoes, as the true culprits. After the link between mosquitoes and their parasites was identified in the early 20th century, the global burden of malaria began to change with the introduction of modern medicine, the development of antimalarial drugs, large-scale efforts in vector control, and drainage of marshy areas. Many countries intensified their malarial control efforts in the mid-20th century, with global malaria eradication campaigns launched by organisations such as the WHO. These efforts focused on controlling mosquito populations through the use of insecticides, along with improved access to antimalarial drugs and public health interventions such as bed nets. One of the insecticides, DDT, was initially celebrated for its effectiveness in interrupting malaria transmission. However, growing evidence in the 1950s and 1960s pointed to serious ecological consequences, including the collapse of bird populations and long-term environmental contamination.[10] These concerns were brought to widespread public attention by the publication of *Silent Spring* in 1962, in which Rachel Carson documented the detrimental impact of DDT ("elixirs of death") use on ecosystems and human health.[11] While the environmental costs of insecticide use ultimately led to restrictions and bans in many countries, the mid-20th-century malaria campaigns did succeed in substantially reducing or even eliminating malaria in most European countries. In 1970, the Netherlands was declared malaria-free following a concerted effort to eliminate the disease through widespread use of insecticides, drainage of marshy areas, and improved sanitation.[12]

From the 1970s to the 1990s, limited progress was made in global malaria control. The emergence and spread of chloroquine-resistant *P. falciparum* strains, particularly in sub-Saharan Africa and Southeast Asia, reduced treatment efficacy and contributed to sustained high levels of morbidity and mortality.[13] During this period, control programmes were often underfunded or fragmented, and malaria remained a major public health burden in endemic regions. In response to the persistent burden of malaria, the Roll Back Malaria partnership was established in 1998 by the WHO, UNICEF, UNDP, and the

World Bank.[14] This initiative aimed to halve malaria deaths by 2010 through coordinated global efforts.[15] Between 2000 and 2015, significant progress was made in reducing malaria incidence and mortality.[16] The widespread distribution of long-lasting insecticide-treated nets, the adoption of artemisinin-based combination therapies (ACTs), and the implementation of indoor residual spraying contributed to these gains. It is estimated that malaria control interventions averted approximately 6 million malaria-related deaths during this period, with the majority of averted cases in sub-Saharan Africa.[17]

Despite substantial progress since 2000, global malaria control has stalled in recent years.[18] This plateau is attributed to a combination of factors, including stagnating funding, coverage of existing tools, and rising resistance to insecticides, drugs, and diagnostics.[18] Additional challenges such as novel vector populations, climate change, conflicts, population displacement, and persistent health system weaknesses-particularly in high–burden countries–have further hindered progress. With an estimated 263 million malaria cases and 597.000 malaria-related deaths in 2023, of which 73.7% occurred in children under five, the global malaria burden remains high.[1]

Biology of malaria and malaria transmission

As mentioned, malaria is caused by parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes. Of the 172 *Plasmodium* species, eight species can infect humans: *P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi*, *P. cynomolgi*, *P. inui*, and *P. fieldi*. *P. falciparum* and *P. vivax* pose the greatest threat to public health, with *P. falciparum* being more prevalent and causing the highest mortality and morbidity.[1] In contrast, *P. vivax* can develop in mosquitoes at lower temperatures and persist at higher altitudes and latitudes, resulting in a broader geographical distribution.[19] Consequently, it accounts for a higher incidence of infections than *P. falciparum* outside of Africa. Furthermore, *P. vivax* also has a dormant liver stage capable of reactivation after months or even years, leading to relapse without requiring new transmission of the parasite.[20]

Plasmodium parasites exhibit a complex life cycle alternating between a female *Anopheles* mosquito vector and the vertebrate human host.[21] Transmission occurs when an infected mosquito bites a human to obtain a blood meal, a process necessary for egg development. To facilitate feeding, the mosquito

injects saliva into the host's skin. This saliva contains anticoagulants that prevent blood clotting, allowing the mosquito to feed more efficiently. If the mosquito is infected with *Plasmodium*, its saliva contains sporozoites that enter the human bloodstream and travel to the liver, initiating infection (**Figure 1**). Inside the liver cells, the parasites undergo rapid replication, forming hepatic schizonts. After several days, these schizonts rupture, releasing thousands of merozoites into the bloodstream and marking the beginning of the erythrocytic (or blood) stage of infection. In the blood, merozoites invade red blood cells, where they develop into ring-stage trophozoites. These trophozoites mature into schizonts that undergo multiple rounds of replication before resulting in the rupture of the infected red blood cell, releasing new merozoites that continue the asexual cycle. In *P. falciparum* infections, this asexual replication cycle recurs approximately every 48 hours and is associated with the recurrent fevers and chills characteristic of malaria. As intra-erythrocytic development progresses, the more mature asexual stages are sequestered in deep vascular beds and are generally absent from peripheral circulation, even in uncomplicated malaria. This sequestration contributes to microvascular obstruction and can lead to severe complications such as cerebral malaria, respiratory distress syndrome, and other organ dysfunction.

Not all parasites continue the asexual cycle. A small proportion differentiate into male and female gametocytes, the sexual stage of the parasite. Gametocytes are not associated with symptoms but are the only parasite life stage that can be transmitted to and establish infection in mosquitoes. In *P. falciparum*, gametocytes develop during five developmental stages over a prolonged 10–12-day maturation period.[22] Immature gametocytes (stages I-IV) sequester outside the peripheral circulation, primarily in the bone marrow and spleen.[23] Only mature stage V gametocytes circulate in peripheral blood,[24] making earlier gametocyte stages undetectable in routine blood smears. The mature stage V gametocytes then become accessible in the peripheral blood for uptake by new blood-feeding mosquitoes.[25] Inside the mosquito's midgut, gametocytes mature into gametes, which fuse to form a zygote (**Figure 1**). The zygote then develops into a motile ookinete that crosses the mosquito's gut wall and forms an oocyst. Within the oocysts, thousands of new sporozoites develop through a process called sporogony. When the oocyst ruptures, the sporozoites migrate to the mosquito's salivary glands, rendering the mosquito infectious. With its next bite, the mosquito injects these sporozoites into a new human host, perpetuating the transmission cycle.

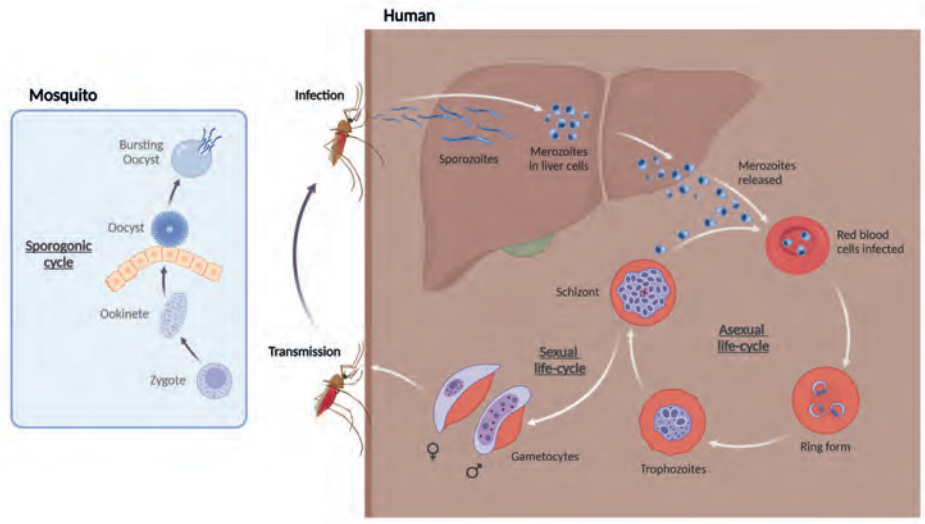

Figure 1. The life cycle of *Plasmodium falciparum. Created with BioRender.com.*

Factors such as the potential for prolonged duration of asymptomatic *P. falciparum* infections in (semi-immune) humans, mosquitoes feeding on multiple hosts, and the efficient transfer of parasites between humans and mosquitoes contribute to reproduction rates (R0) for malaria exceeding 100 in many African regions.[26] Interrupting human-to-mosquito transmission is therefore an important element in the effort towards malaria elimination. One of the challenges in malaria control is that many infected individuals remain asymptomatic or undiagnosed, making them unaccounted for within the health system.[27] These people unwittingly contribute to the cycle of *P. falciparum* transmission by producing gametocytes that infect mosquitoes. Effective disease control and elimination strategies will need to take into account this large "infectious reservoir". The population most responsible for sustaining malaria transmission is not necessarily the same as the population with the highest incidence of clinical disease. Depending on the local epidemiological context, different age-groups–such as older children or adults–may contribute most to onward transmission. In many endemic settings children under 5 years of age bear the brunt of malaria morbidity and mortality, whereas school-age children (aged 5-15 years) are particularly relevant drivers of malaria transmission. This population often carries parasites at higher densities and for longer periods than younger children,[28,29] whilst also being disproportionally bitten by mosquitoes due to their larger body size and lower coverage with insecticide treated nets.[30] To reduce the efficient transmission of *P. falciparum*, various new tools and approaches will be explored in this thesis. As with

many infectious diseases, effective treatment of infection is not only a way to prevent (severe) clinical consequences for the infected individual but also a way to prevent onward transmission.

Malaria treatment

The past and present

Quinine, the first effective antimalarial drug, is derived from the bark of the cinchona tree—also known as the 'fever tree' (l'arbre des fièvres)—native to South America. Known in Europe as "Jesuit's powder" or "Peruvian bark", its therapeutic use against fevers was introduced via Jesuit missionaries in the 17th century.[31] Nearly two centuries passed before the active compounds of the cinchona bark were successfully isolated in 1820 by two French chemists. Recognising their medicinal and commercial value, the Dutch government began cultivating 20,000 *Cinchona ledgeriana* trees on Java in 1865. By the end of the 19th century, the Netherlands had established a near-monopoly on quinine production, supplying over 90% of the world's supply.[32] Owing to quinine's intensely bitter taste, it has been rumoured that the British allegedly mixed water, sugar, and gin with their quinine to make it more palatable, thus inventing the famous gin and tonic drink.

The development of chloroquine in the 1930s marked a turning point in antimalarial therapy. The synthetic four-aminoquinoline chloroquine proved highly effective, well tolerated, and inexpensive, and became the cornerstone of global malaria control after the World War II. However, resistance emerged by the late 1950s in Southeast Asia and South America, eventually spreading to Africa and undermining its effectiveness.[13] Subsequent advances in antimalarial therapy included the introduction of sulfadoxine-pyrimethamine in the late 1960s.[33] Although sulfadoxine-pyrimethamine had limited utility for the treatment of clinical malaria due to the rapid emergence of resistance –initially in Southeast Asia and later across Africa–[34] it remains in use for preventive purposes. Sulfadoxine-pyrimethamine is widely used as intermittent preventive treatment during pregnancy to reduce adverse maternal and neonatal outcomes, and in combination with amodiaquine it is used for seasonal malaria chemoprevention (SMC) in young children living in highly seasonal transmission areas.[1]

In response to the emergence of resistance to both chloroquine and quinine, researchers at the Walter Reed Army Institute developed mefloquine, a 4-quinoline methanol. Its efficacy in preventing *P. falciparum* malaria was first reported in 1974,[35] and it was subsequently shown to be an effective treatment.[36] However, as with previous antimalarials, resistance to mefloquine began to emerge, with clinical evidence first reported in Asia around 1985,[37] coinciding with its broader deployment.

The re-discovery of artemisinin in 1972 by Chinese scientists marked a major breakthrough in the fight against drug-resistant malaria.[38] Isolated from *Artemisia annua* (sweet wormwood), a plant long used in traditional Chinese medicine, artemisinin has a unique chemical structure and rapid parasite clearance. It forms the basis of artemisinin-based combination therapies (ACTs), now considered the frontline treatment for *P. falciparum* malaria in many endemic regions. The most common derivatives of artemisinin are artemether, artesunate and dihydroartemisinin. ACTs pair a short-acting artemisinin derivative with a long-acting partner drug (such as lumefantrine, piperaquine, or amodiaquine). ACTs help prevent resistance to the individual drugs by combining two drugs with different mechanisms of action.[39]

Antimalarial strategies to reduce *P. falciparum* transmission

In malaria control and elimination programs, antimalarial drugs are used not only to cure infections but also to reduce human-to-mosquito transmission through various strategies. First, prompt treatment of symptomatic individuals with effective ACTs not only cures disease but also reduces infectivity to mosquitoes by clearing asexual parasites and early-stage gametocytes, thereby halting the ongoing development of transmissible mature gametocytes.[40] In high-transmission settings, however, symptomatic individuals represent only a small fraction of the parasite-positive population. To further reduce the infectious reservoir, broader drug-based interventions are needed.

Mass drug administration (MDA) involves treating entire populations or at-risk groups without knowledge of their individual infection status, aiming to clear both symptomatic and asymptomatic infections en masse. When implemented with high coverage, MDA can rapidly reduce prevalence and interrupt local transmission, particularly in low-transmission or pre-elimination settings.[41] WHO recommends MDA in specific contexts such as epidemics, elimination campaigns, or containment of drug resistance.[41]

Its effectiveness depends on adherence, regimen selection (often ACT plus single-low dose primaquine), and integration with surveillance and vector control. However, without follow-up measures, transmission can quickly rebound.

Between individual treatment and MDA lies a range of possible targeted strategies aimed at those individuals most likely to sustain transmission. In many settings, school-aged children carry the highest prevalence of asymptomatic *P. falciparum* infections and are highly infectious to mosquitoes.[29] Targeting such subpopulations–through interventions like school-based treatment or seasonal campaigns–can reduce community transmission without requiring treatment of the entire population.[42]

Each of these drug-based strategies–individual treatment, MDA, and targeted approaches–has distinct value depending on the epidemiological context. They are often combined to maximise impact, with the shared goal of reducing the infectious reservoir and limiting onward transmission. When implemented alongside vector control and surveillance, they form a cornerstone of malaria elimination efforts.

Gametocytocidal activity of artemisinin-based combination therapies

The primary aim in the therapeutic management of malaria is the clearance of pathogenic asexual blood-stage parasites using antimalarials with schizonticidal properties. ACTs are the first-line treatment for uncomplicated *P. falciparum* malaria across the world and are highly potent against asexual parasites. They have different characteristics in terms of dosing schedules, the speed at which they clear the parasite biomass, and the duration of additional chemoprophylaxis they offer. However, *P. falciparum* gametocytes have markedly different sensitivity profiles to antimalarial drugs than asexual parasites.

After clearance of asexual parasites, which form the source of gametocyte production, the duration of ongoing gametocyte carriage is determined by the period of gametocyte sequestration and the time they remain in circulation after entering the bloodstream. Sequestration has been estimated to last up to 12 days,[43] while circulating gametocytes may persist for as long as 22.2 days.[43] After non-ACT treatment, individuals can carry gametocytes for up to 3-6 weeks following clearance of asexual parasites.[44] ACT shortens this period

of gametocyte carriage considerably with the exact effect depending on the ACT of choice.[44]

Artemisinin derivates in ACTs clear immature gametocytes, but have limited direct impact on mature gametocytes.[40] Most partner drugs in ACTs (e.g. lumefantrine, amodiaquine, piperaquine) similarly show only modest or no activity against mature stage V gametocytes.[45] As a result, the reduction in gametocyte carriage with ACT is mostly due to elimination of asexual stages (preventing new gametocyte formation) rather than killing of existing mature gametocytes.[46] However, assessing post-treatment transmission potential based solely on gametocyte carriage is insufficient, as transmissible gametocytes may persist at submicroscopic levels and some antimalarial drugs can sterilise gametocytes prior to their clearance from circulation. Accurate evaluation therefore requires mosquito feeding assays to directly measure infectivity before and after treatment. Enhancing standard antimalarial treatments or mass drug administration campaigns with a gametocytocidal agent may accelerate malaria elimination efforts and help contain the spread of artemisinin-resistant *P. falciparum* strains. To inform specific treatment recommendations, it is essential to know how different antimalarial regimens impact transmission potential. In **Chapters 2, 3A, and 4**, we explore how different ACTs and the non-ACT sulfadoxine-pyrimethamine plus amodiaquine affect gametocyte carriage and how this translates into transmission reduction.

The 8-aminoquinolines: primaquine and tafenoquine

Primaquine is an 8-aminoquinoline used since the 1950s as the only widely available drug to kill dormant liver stages (hypnozoites) of *P. vivax* and *P. ovale* and achieve radical cure, but it also has activity against (mature) *P. falciparum* gametocytes.[47,48] Its main limitation is dose-dependent haemolytic toxicity in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, a common condition in malaria-endemic regions. For radical cure of *P. vivax*, high daily doses of 3.5 mg/kg primaquine are used over 14 days and in those cases G6PD screening is required. However, a single low dose of 0.25 mg/kg primaquine is sufficient to kill gametocytes, is considered safe without prior G6PD testing,[49] and is recommended by WHO in combination with ACT for confirmed *P. falciparum* cases to reduce onward transmission.[48]

It remains unclear whether adding primaquine to artesunate-amodiaquine or artemether-lumefantrine–the most widely used treatment regimen for uncomplicated *P. falciparum* infections globally–[50] offers additional benefit,

or whether these ACTs alone already sufficiently prevent post-treatment transmission. In **Chapters 3A and 4**, we assessed artemether-lumefantrine and artesunate-amodiaquine with and without a single low-dose of primaquine for *P. falciparum* transmission reduction.

Tafenoquine is a newer, long-acting 8-aminoquinoline developed as a single-dose alternative to primaquine for radical cure of *P. vivax*.[51] Like primaquine, it targets hypnozoites and gametocytes, but its much longer half-life (15 days versus 4-9 hours for primaquine) allows for a simplified dosing regimen and improved compliance.[52] Given its similar mechanism of action and toxicity profile, G6PD testing remains required before its use.[53] Tafenoquine's potential to reduce *P. falciparum* transmission remains largely unexplored. Therefore, in **Chapters 2 and 3A**, we assessed the gametocytocidal potential of tafenoquine. In **Chapter 2**, we tested different single low doses in combination with an ACT, while in **Chapter 3A**, we evaluated a single low dose together with a non-ACT regimen.

Treatment of uncomplicated malaria: the constant battle with resistance

The emergence and spread of partial resistance to artemisinin derivatives in Southeast Asia[54,55] and more recently in East Africa[56,57] pose a threat to malaria control, with the potential to increase both malaria incidence and mortality. To counter this, antimalarial strategies aimed at slowing resistance development are needed. One such strategy involves combining more than two drugs–an approach successfully employed in the treatment of tuberculosis and HIV, diseases also characterised by high resistance potential. This includes novel combinations of existing drugs or the addition of drugs that specifically target gametocytes. One such approach is the use of triple artemisinin-based combination therapies (TACTs) that combine a standard ACT with a second, slowly eliminated partner drug to enhance parasite clearance and delay resistance emergence.[58] Artemether-lumefantrine-amodiaquine is one such TACT that has been shown to be safe, well tolerated, and effective in treating uncomplicated *P. falciparum* malaria in Asia, including in regions with known resistance to artemisinin and its partner drugs.[59,60] However, the extent to which artemether-lumefantrine-amodiaquine affects mature gametocytes and thereby influences transmission potential to mosquitoes remains unclear. In **Chapter 4**, we aimed to address this question.

Mosquito feeding assays

Infectiousness of gametocytes

To determine whether a drug or vaccine reduces *P. falciparum* transmission, a validated assay is required. Detecting the presence of gametocytes alone is not sufficient, as the infectiousness of gametocytes is influenced by multiple factors: gametocyte density,[61] sex-ratio,[62] gametocyte maturity,[63] the duration of infection,[64] intrinsic parasite factors,[65,66] human genetic factors,[67] and human clinical and immune responses.[68-70] As such, gametocyte detection alone does not provide a reliable measure of an individual's transmission potential. Mosquito feeding assays are widely used to quantify malaria transmission potential in both epidemiological and clinical studies. These assays typically assess the infection status of blood-fed mosquitoes by detecting or quantifying the number of established oocysts on the mosquito midgut approximately seven days post-infection.

To evaluate the transmission-reducing effect of drugs or vaccines, several mosquito feeding assays are available. These include the direct skin feeding assay (DSF), the direct membrane feeding assay (DMFA), and the standard membrane feeding assay (SMFA).

Field-based mosquito feeding assays: direct skin feeding and direct membrane feeding

DSF and DMFA are field-based assays commonly used to determine infectiousness of *P. falciparum* in naturally infected gametocyte carriers. In DSF, laboratory-reared, *P. falciparum*-free mosquitoes feed directly on the blood of a naturally infected individual through the skin (**Figure 2**). Following dissection of the mosquito midgut approximately 7 days after feeding, oocysts can be detected to confirm infection. In the DMFA, blood is collected from naturally infected individuals with gametocytes and, following a washing step, is offered to mosquitoes either with the participant's own plasma or with malaria-naïve control serum (**Figure 2**).

DSF and DMFA allow for gametocyte quantification and the use of naturally circulating parasite strains and locally caught and reared mosquitoes. DSF most closely mimics natural transmission conditions and generally results in a higher proportion of infected mosquitoes.[71] The DMFA offers a slightly more controlled alternative and can also be used in preclinical settings to assess the efficacy of test sera in combination with naturally circulating gametocytes.[72]

The DMFA further allows the inclusion of large numbers of mosquitoes, which increases the precision of transmission estimates, and is more appropriate for repeated assays. In both DSF and DMFA, the proportion of infected mosquitoes is typically used as the primary outcome measure, referred to as transmission-blocking activity (TBA), defined as the percentage reduction in the proportion of infected mosquitoes when comparing experimental with control conditions.

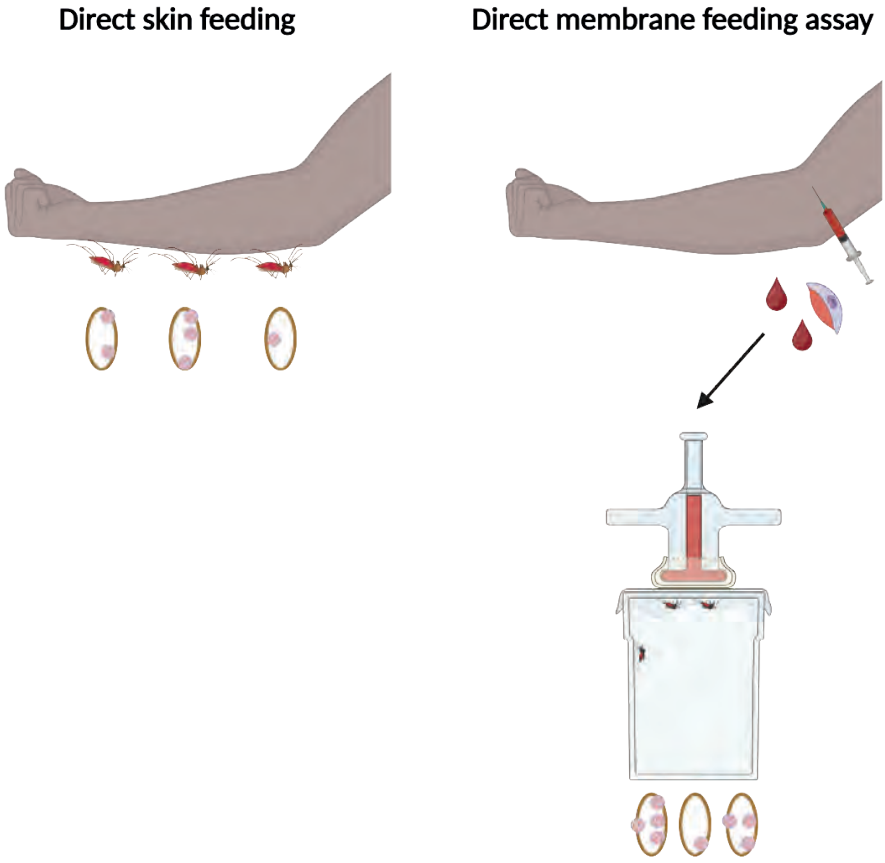

Figure 2. Field-based mosquito feeding assays. In direct skin feeding assays, mosquitoes are placed directly on the skin of a parasitaemic host to feed. Direct membrane feeding assays involve offering mosquitoes a blood sample obtained via venepuncture or finger prick from a naturally infected individual. With direct membrane feeding assays, it is moreover possible to replace autologous plasma from the gametocyte donor's blood with (malaria-naïve) control serum. The blood is presented using water-jacketed glass feeders maintained at approximately 37°C, with mosquitoes feeding through a membrane. Oocysts can be detected on the mosquito midgut after dissection of the mosquito. *Created with BioRender.com*

Standard membrane feeding assay

An important complementary tool is the SMFA, which is well suited for preclinical evaluation. In this assay, *in vitro* cultured *P. falciparum* gametocytes are mixed with test serum and fed through membrane feeders to a laboratory-reared colony of uninfected *Anopheles* mosquitoes under highly standardised conditions (**Figure 3**).[73] The SMFA is specifically optimised for high infection efficiency to ensure robust readouts and minimise day-to-day and batch variation.[74] Because the assay is designed for high mosquito infection rates, the proportion of infected mosquitoes (TBA) becomes less informative, and transmission-reducing activity (TRA) is generally preferred as the primary outcome. TRA is calculated as the %-reduction in the number of oocysts in mosquitoes fed on gametocytes in the presence of participants' serum compared with mosquitoes fed on gametocytes in the presence of control serum. Due to lower precision associated with low TRA estimates and based on the historical threshold used to support clinical development of transmission-blocking vaccines, >80% TRA is often set as the efficacy threshold of interest.[71,75]

One of the key advantages of the SMFA is that it allows for titration experiments, repeat testing, and greater control over experimental conditions compared to field assays, which are often limited by logistical and ethical constraints. On the other hand, SMFA can only use a limited number of laboratory-adapted parasite strains and artificial feeding conditions that do not fully reflect natural transmission dynamics.

SMFA, DMFA, and DSF are best viewed as complementary assays. While the relationship between their respective outcome measures remain an active area of research, they collectively offer a comprehensive toolkit for evaluating the functional efficacy of transmission-blocking interventions. In **Chapters 2, 3A and 4**, we employ DMFA to determine the transmission-reducing efficacy of different antimalarial combinations. In **Chapters 5 and 6** the SMFA is used to evaluate the transmission-reducing activity of the R0.6C vaccine and the TB31F monoclonal antibody.

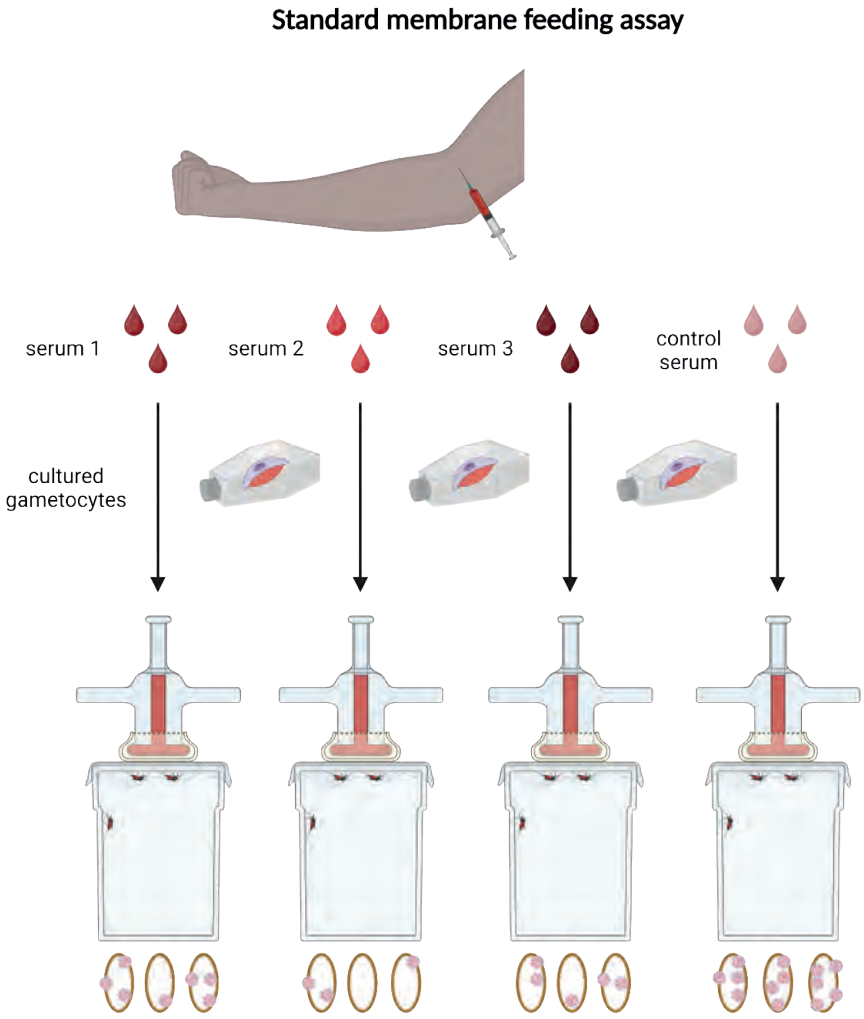

Figure 3. Standard-membrane feeding assay. In the SMFA, cultured gametocytes with either participant or control serum are offered to mosquitoes. The infection prevalence or intensity in mosquito batches fed with control serum is then compared to that in batches fed with participant serum. The SMFA allows for the simultaneous evaluation of a large number of participant sera. *Created with BioRender.com.*

Parasite antigens as targets of natural and vaccine-induced immunity inside the mosquito

P. falciparum expresses a distinct set of antigens during its sexual development that play key roles in fertilisation and parasite development within the mosquito. Among the most studied are Pfs48/45 and Pfs230, both

members of the 6-cysteine protein family,[76] that are essential for successful gamete fusion.[77,78] These proteins are located on the surface of gametes in the mosquito midgut and are highly conserved, making them attractive targets for transmission-blocking interventions.

Functionally, antibodies directed against Pfs48/45 and Pfs230 can interfere with fertilisation and block the development of the parasite within the mosquito. While these proteins are not accessible to the immune system during their expression in intra-erythrocytic gametocytes, they become immunogenic when gametocytes die and are processed by the spleen, which leads to the generation of systemic antibody responses.[22]

Evidence from natural infections shows that these antigens can be targets of naturally acquired immunity. Antibodies against Pfs230 and Pfs48/45 are associated with TRA in both SMFA and DMFA.[79] However, high antibody titres are not always predictive of functional activity, suggesting the importance of antibody quality and epitope specificity.[79] Additionally, responses to a broader set of gametocyte antigens beyond Pfs230 and Pfs48/45 contribute to TRA,[79] indicating that natural exposure generates a polyclonal response targeting multiple proteins, some of which may not yet be functionally characterised.

These insights support the development of vaccines that either amplify naturally acquired responses (e.g. targeting Pfs230 or Pfs48/45)[69] or induce novel functional immunity independent of natural exposure, such as with Pfs25, an antigen expressed only after fertilisation in the mosquito and typically not immunogenic during natural infection.[80]

Lead targets of transmission-blocking vaccines

Transmission-blocking vaccines aim to induce antibodies against such antigens, that are taken up by the mosquito vector together with the infectious blood meal containing gametocytes. In the mosquito midgut these antibodies bind to these surface antigens on sexual stage parasites and interfere with parasite fertilisation and sexual development. Unlike traditional vaccines, transmission-blocking vaccines do not directly protect the vaccinated individual. Instead, they function by inhibiting parasite development within the mosquito, thereby reducing onward transmission. Currently, the following three sexual stage antigens are under clinical development and are leading transmission-blocking vaccine candidates: Pfs25, Pfs230, and Pfs48/45.[80,81]

Pfs25 is the most extensively studied transmission-blocking vaccine candidate, partly due to the relative ease of protein expression.[82] While preclinical studies have consistently shown Pfs25 to be an effective transmission-blocking antigen,[83,84] clinical trials have revealed limitations in inducing potent and durable antibody responses in humans.[85-87] These findings raised questions about the viability of further Pfs25-based vaccine development, particularly after a clinical trial directly comparing Pfs25- and Pfs230-based vaccines showed that Pfs230-based vaccine elicited stronger TRA.[88] For Pfs230, early observations showed that a recombinant protein fragment containing the N-terminal region of the protein was able to induce functional antibodies.[89] Pfs230-based vaccines have since advanced in clinical testing, with multiple studies conducted in both malaria-naïve and malaria-exposed populations.[88,90] However, Pfs230 vaccine has shown inconsistent immunogenicity, with some participants developing functional antibodies.[88]

Pfs48/45-based transmission-blocking vaccine

The sexual stage antigen Pfs48/45 has long been recognised as a promising target for malaria transmission-blocking vaccines. In the early 1980s, Pfs48/45 was identified as a gamete surface protein capable of inducing transmission-blocking antibodies,[91] and subsequent studies showed that specific monoclonal antibodies targeting conformational epitopes on this protein could prevent oocyst formation in mosquitoes.[92] Despite its early promise, the clinical development of a Pfs48/45-based vaccine was hampered by difficulties in achieving high yields of properly folded recombinant protein capable of inducing functional antibodies.[93] Progress was made with the fusion of two Pfs48/45 domains (10C) to the N-terminal region of GLURP (R0), but the yields remained suboptimal.[94] The expression of a single domain of Pfs48/45 (6C) improved expression levels significantly[95] and paved the way for production of the transmission-blocking vaccine R0.6C for clinical testing.[96] In **Chapter 5**, we evaluate the safety and transmission-blocking activity of the first Pfs48/45 based transmission-blocking vaccine R0.6C in malaria-naïve individuals.

Monoclonal antibodies as a novel tool for the malaria elimination toolbox

The effectiveness of transmission-blocking vaccines ultimately depends on their ability to induce and maintain high levels of functional antibodies targeting key parasite antigens in a large proportion of the vaccinated individuals.[88]

However, the titres required to achieve functional TRA vary depending on the specific antigen and individual variability in immune responses. One challenge is generating antibody levels that are both sufficiently high and durable to provide an extended period of effective TRA–ideally long enough to cover at least one full transmission season. In addition to optimising vaccine components and enhancing formulations with adjuvants, an alternative strategy is the direct administration of monoclonal antibodies. Rather than functioning as vaccines, transmission-reducing monoclonal antibodies act more like therapeutic agents: they provide immediate passive immunity. They can be strategically administered–for instance, ahead of a transmission season to suppress the high basic reproduction rate (R_0) early on, or in combination with antimalarial chemotherapy to prevent the spread of resistant parasites. Monoclonal antibodies are already used in the prevention and treatment of various infectious diseases, including in paediatric populations.[97-99] The high efficacy of the monoclonal antibody CIS43LS in preventing malaria infection in a human challenge model highlights their potential as a complementary tool in malaria control,[100] which we discuss further in **Chapter 7**.

Pfs48/45-based transmission-reducing monoclonal antibody

The most potent transmission-reducing monoclonal antibody described to date is 85RF45.1, a rat-derived antibody obtained after immunisation with *P. falciparum* NF54 gametocytes.[101] This antibody recognises a highly conserved epitope on Pfs48/45, the same antigen targeted by the transmission-blocking vaccine candidate R0.6C. 85RF45.1 has been humanised into TB31F, retaining its original binding characteristics and potency.[102] Humanisation is the process of modifying a non-human antibody to make its amino acid sequence in the constant regions more similar to that of human antibodies, thereby reducing the risk of anti-antibody immune reactions when administered in humans. A safe and efficacious transmission-reducing monoclonal antibody for use in humans could be a valuable addition to the malaria elimination toolbox, especially when combined with other control measures. In **Chapter 6**, we evaluate the safety, tolerability, and potential *P. falciparum* transmission-reducing activity of the monoclonal antibody TB31F in humans.

Malaria research in the era of climate change

All current public health challenges and interventions occur against the backdrop of another phenomenal challenge that affects life on earth. Climate change is widely recognised as one of the most pressing global health threats of our time. Its impacts on human well-being are far-reaching and unequally distributed, with vulnerable populations bearing the greatest burden. Among its many consequences, climate change is expected to alter the distribution and burden of infectious diseases.[103] Vector-borne diseases are driven by environmental and climatic factors, making them sensitive to climate change.[104-106] Among these diseases, malaria contributes the highest burden globally, raising concerns about how climate change will affect progress against the disease.[107] Because malaria transmission is greatly influenced by climatic factors such as temperature, rainfall, and humidity,[105,108,109] even relatively small changes in local climate conditions can influence the geographical range, seasonality, and intensity of transmission.[110,111] In addition to these direct effects, climate change can also affect malaria risk indirectly–by exacerbating food insecurity, increasing population displacement, and disrupting access to essential health services and commodities.[103,107]

To sustain progress against malaria, both climate adaptation and mitigation will be needed. Climate adaptation measures–including strengthened surveillance, vector control in newly affected regions, and health system preparedness for extreme weather events–are critical to managing shifting malaria risk in areas where the disease has not historically been endemic.[112] At the same time, mitigation efforts that aim to reduce greenhouse gas emissions are essential to limit long-term increases in transmission potential and global health inequities.

The health sector itself is a significant contributor to climate change, accounting for an estimated 5.2% of global greenhouse gas emissions–with even higher proportions in some high-income countries.[113] Within this sector, medical research and clinical trials are often overlooked sources of emissions, despite their frequent reliance on energy-intensive infrastructure, international travel, temperature-controlled logistics, and high volumes of disposable materials. These activities carry a notable environmental burden, which challenges the ethical imperative of the medical profession to "do no harm"–not only to patients, but also to the planet. As research funders and institutions increasingly commit to climate action, there is growing recognition

that scientific research itself must be critically examined for its environmental footprint and reimagined with sustainability in mind.

With growing urgency to tackle both climate change and global health inequities, there is increasing focus on understanding and reducing the environmental impact of medical research. However, to date, the sparse studies in this field have almost exclusively focused on calculating the carbon footprint of specific activities, typically limited to estimating greenhouse gas emissions. While this offers valuable insights, it captures only a part of the overall environmental impact associated with research activities. A life cycle assessment (LCA), by contrast, can provide a more comprehensive and detailed picture. An LCA is a method for evaluating the environmental impact of a product, process, or service across all stages of its life cycle—from raw material extraction and production, through use, to final disposal. Unlike a standard carbon footprint analysis, which focuses solely on greenhouse gas emissions, an LCA takes a broader perspective by including a range of environmental indicators such as energy consumption, water use, resource depletion, waste generation, and various pollution metrics. This makes LCA particularly useful for identifying environmental "hotspots" and trade-offs that might otherwise remain hidden. To our knowledge, no LCA has yet been conducted for a clinical trial, which could provide a more complete picture of the trial's environmental footprint and help identify key contributors to environmental impact. To address this gap, **Chapter 3B** presents an LCA of a clinical malaria trial conducted in Mali. This study complements the main findings of this trial, presented in **Chapter 3A.**

Expanding vaccine research

While reducing the transmission of *P. falciparum* is the central focus of this thesis, the broader implications of vaccine development extend beyond this single disease. Infectious diseases continue to pose threats to global health, as demonstrated by the COVID-19 pandemic. The rapid development and deployment of vaccines during the pandemic underscored the importance of flexible, scalable, and adaptable vaccine platforms. Novel technologies, such as capsid virus-like particle (cVLP) vaccines, offer promising solutions for a range of infectious diseases. cVLP-based vaccines mimic the structure of a virus but lack genetic material, making them non-infectious while still being capable of eliciting strong, broad, and long-lasting immune responses. cVLP

vaccines have been successfully marketed and have demonstrated long-term effectiveness, as with vaccines against human papillomavirus.[114] One of the major advantages of VLPs is their ability to enhance antigen presentation, prolong the immune response, and offer a modular system for rapid adaptation to new pathogens.

During the COVID-19 pandemic, a capsid virus-like particle (cVLP) vaccine platform, originally intended to improve malaria vaccines,[115] was developed towards human use for SARS-CoV-2: ABNCoV2. **Chapter 8** explores whether this cVLP vaccine can enhance immune responses compared to conventional COVID-19 vaccines, particularly in terms of durability and breadth of protection against viral variants. The insights gained from this study contribute to the broader understanding of vaccine development and could be applied to future malaria vaccine efforts. By examining how novel vaccine platforms perform in human trials, we can gain a broader perspective on the future of infectious disease prevention and demonstrate how technological advancements in one field can inform progress in another.

Aims and outline of this thesis

To support malaria elimination efforts, this thesis focuses on interventions that reduce *Plasmodium falciparum* transmission. The work is divided into three parts that aim to: i) evaluate the transmission-reducing potential of antimalarial drugs, ii) explore active and passive immunisation strategies to interrupt transmission, and iii) broaden the scope of clinical vaccine research beyond malaria.

Objectives Part 1: Unveiling the *Plasmodium falciparum* transmission-reducing potential of antimalarials

- To establish the efficacy and safety of three single low doses of tafenoquine in combination with dihydroartemisinin–piperaquine for reducing gametocyte density and transmission to mosquitoes (Chapter 2)
- To determine the efficacy of artemether–lumefantrine with and without primaquine and sulfadoxine–pyrimethamine plus amodiaquine with and without tafenoquine for reducing gametocyte carriage and transmission to mosquitoes (Chapter 3A)
- To examine the environmental impact of a clinical malaria trial to better understand the interplay between various environmental impact categories

and make informed decisions that promote sustainable research practices (Chapter 3B)
- To determine the safety and efficacy of artemether–lumefantrine–amodiaquine and artesunate–amodiaquine with and without single low-dose primaquine for reducing gametocyte carriage and transmission to mosquitoes (Chapter 4)

Objectives Part 2: Active and passive immunisation to reduce *Plasmodium falciparum* transmission
- To assess the safety and transmission-reducing efficacy of R0.6C adsorbed to aluminum hydroxide with and without Matrix-M™ adjuvant in malaria-naïve individuals (Chapter 5)
- To evaluate the safety and transmission-reducing efficacy of the monoclonal antibody TB31F in malaria-naive participants (Chapter 6)
- To reflect on the potential of monoclonal antibodies for malaria prevention and discuss key considerations for their implementation (Chapter 7)

Objectives Part 3: Beyond malaria: expanding vaccine research
- To clinically test a modular cVLP COVID-19 vaccine in SARS-CoV-2-naïve adults (Chapter 8)

References

1. World Health Organization. World malaria report 2024: addressing inequity in the global malaria response. Geneva, 2024.
2. Hawass Z, Gad YZ, Ismail S, et al. Ancestry and pathology in King Tutankhamun's family. *JAMA* 2010; **303**(7): 638-47.
3. Sallares R. Malaria and Rome: A History of Malaria in Ancient Italy: Oxford University Press; 2002.
4. Bruce-Chwatt LJ. John Macculloch, M.D., F.R.S. (1773-1835) (The precursor of the discipline of malariology). *Med Hist* 1977; **21**(2): 156-65.
5. Sinton JA. What Malaria Costs India, Nationally, Socially and Economically: Thacker's Press & Directories; 1935.
6. Öberg S, Rönnbäck, K. Mortality among European settlers in pre-colonial West Africa: The "White Man's Grave" revisited. Göteborg; 2016.
7. Fiammetta R. The Miraculous Fever-tree: Malaria and the Quest for a Cure that Changed the World: HarperCollins; 2003.
8. Mertens JE. A History of Malaria and Conflict. *Parasitol Res* 2024; **123**(3): 165.
9. Shanks D, Karwacki JJ. Malaria as a military factor in Southeast Asia. *Mil Med* 1991; **156**(12): 684-6.
10. Newton I. Population Ecology of Raptors; 1979.
11. Carson R. Silent Spring; 1962.
12. World Health Organization. Countries and territories certified malaria-free by WHO. 2025. https://www.who.int/teams/global-malaria-programme/elimination/countries-and-territories-certified-malaria-free-by-who (accessed 14 March 2025).
13. Wellems TE, Plowe CV. Chloroquine-resistant malaria. *J Infect Dis* 2001; **184**(6): 770-6.
14. Malaria is alive and well killing more than 3000 African children every day. WHO and UNICEF call for urgent increased effort to roll back malaria. *Indian J Med Sci* 2003; **57**(5): 215-6.
15. Remme JH, Binka F, Nabarro D. Toward a framework and indicators for monitoring Roll Back Malaria. *Am J Trop Med Hyg* 2001; **64**(1-2 Suppl): 76-84.
16. World Health Organization. World Malaria Report 2015. Geneva, Switzerland, 2015.
17. World Health Organization. WHO commends the Roll Back Malaria Partnership's contribution to global progress as governing board disbands secretariat. 2015.
18. World Health Organization. World malaria report 2020: 20 years of global progress and challenges. Geneva, 2020.
19. 6. The Parasite, the Mosquito, and the Disease. In: Arrow KJ, Panosian C, Gelband H, eds. Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance. Washington (DC); 2004.
20. White NJ. Determinants of relapse periodicity in Plasmodium vivax malaria. *Malar J* 2011; **10**: 297.
21. Cowman AFH, J.; Marapana, D.; Marsh, K. Malaria: Biology and Disease. *Cell* 2016; **167**: 610-24.
22. de Jong RM, Tebeje SK, Meerstein-Kessel L, et al. Immunity against sexual stage Plasmodium falciparum and Plasmodium vivax parasites. *Immunol Rev* 2020; **293**(1): 190-215.

23. Joice R, Nilsson SK, Montgomery J, et al. Plasmodium falciparum transmission stages accumulate in the human bone marrow. *Sci Transl Med* 2014; **6**(244): 244re5.
24. Aguilar R, Magallon-Tejada A, Achtman AH, et al. Molecular evidence for the localization of Plasmodium falciparum immature gametocytes in bone marrow. *Blood* 2014; **123**(7): 959-66.
25. Pelle KG, Oh K, Buchholz K, et al. Transcriptional profiling defines dynamics of parasite tissue sequestration during malaria infection. *Genome Med* 2015; **7**(1): 19.
26. Johnston GL, Smith DL, Fidock DA. Malaria's missing number: calculating the human component of R0 by a within-host mechanistic model of Plasmodium falciparum infection and transmission. *PLoS Comput Biol* 2013; **9**(4): e1003025.
27. World Health Organization. Global technical strategy for malaria 2016-2030, 2015.
28. Rek J, Blanken SL, Okoth J, et al. Asymptomatic School-Aged Children Are Important Drivers of Malaria Transmission in a High Endemicity Setting in Uganda. *J Infect Dis* 2022; **226**(4): 708-13.
29. Andolina C, Rek JC, Briggs J, et al. Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. *Lancet Infect Dis* 2021; **21**(11): 1568-78.
30. Stone W, Goncalves BP, Bousema T, Drakeley C. Assessing the infectious reservoir of falciparum malaria: past and future. *Trends Parasitol* 2015; **31**(7): 287-96.
31. Harrison N. In celebration of the Jesuit's powder: a history of malaria treatment. *The Lancet Infectious Diseases* 2015; **15**(10): 1143.
32. Goss A. Building the world's supply of quinine: Dutch colonialism and the origins of a global pharmaceutical industry. *Endeavour* 2014; **38**(1): 8-18.
33. Bloland PB, Lackritz EM, Kazembe PN, Were JB, Steketee R, Campbell CC. Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *J Infect Dis* 1993; **167**(4): 932-7.
34. Wernsdorfer WH, Payne D. The dynamics of drug resistance in Plasmodium falciparum. *Pharmacol Ther* 1991; **50**(1): 95-121.
35. Rieckmann KH, Trenholme GM, Williams RL, Carson PE, Frischer H, Desjardins RE. Prophylactic activity of mefloquine hydrochloride (WR 142490) in drug-resistant malaria. *Bull World Health Organ* 1974; **51**(4): 375-7.
36. Trenholme CM, Williams RL, Desjardins RE, et al. Mefloquine (WR 142,490) in the treatment of human malaria. *Science (80-)* 1975; **190**(4216): 792-4.
37. Hoffman SL, Rustama D, Dimpudus AJ, et al. RII and RIII type resistance of Plasmodium falciparum to combination of mefloquine and sulfadoxine/pyrimethamine in Indonesia. *Lancet* 1985; **2**(8463): 1039-40.
38. Klayman DL. Qinghaosu (artemisinin): an antimalarial drug from China. *Science (80-)* 1985; **228**(4703): 1049-55.
39. White N. Antimalarial drug resistance and combination chemotherapy. *Philos Trans R Soc Lond B Biol Sci* 1999; **354**(1384): 739-49.
40. Group WGS. Gametocyte carriage in uncomplicated Plasmodium falciparum malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data. *BMC Med* 2016; **14**: 79.
41. World Health Organization. Mass drug administration for falciparum malaria: a practical field manual. Geneva, 2017.
42. Sturrock HJ, Hsiang MS, Cohen JM, et al. Targeting asymptomatic malaria infections: active surveillance in control and elimination. *PLoS Med* 2013; **10**(6): e1001467.

43. Eichner M, Diebner HH, Molineaux L, Collins WE, Jeffery GM, Dietz K. Genesis, sequestration and survival of Plasmodium falciparum gametocytes: parameter estimates from fitting a model to malariatherapy data. *Trans R Soc Trop Med Hyg* 2001; **95**(5): 497-501.
44. Bousema T, Okell L, Shekalaghe S, et al. Revisiting the circulation time of Plasmodium falciparum gametocytes: molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. *Malar J* 2010; **9**: 136.
45. Lelievre J, Almela MJ, Lozano S, et al. Activity of clinically relevant antimalarial drugs on Plasmodium falciparum mature gametocytes in an ATP bioluminescence "transmission blocking" assay. *PLoS One* 2012; **7**(4): e35019.
46. Bousema JT, Schneider P, Gouagna LC, et al. Moderate effect of artemisinin-based combination therapy on transmission of Plasmodium falciparum. *J Infect Dis* 2006; **193**(8): 1151-9.
47. Eziefula AC, Bousema T, Yeung S, et al. Single dose primaquine for clearance of Plasmodium falciparum gametocytes in children with uncomplicated malaria in Uganda: a randomised, controlled, double-blind, dose-ranging trial. *Lancet Infect Dis* 2014; **14**(2): 130-9.
48. World Health Organization. Global Malaria Programme: WHO policy brief on single-dose primaquine as gametocytocide in *Plasmodium falciparum* malaria, 2015.
49. Bastiaens GJH, Tiono AB, Okebe J, et al. Safety of single low-dose primaquine in glucose-6-phosphate dehydrogenase deficient falciparum-infected African males: Two open-label, randomized, safety trials. *PLoS One* 2018; **13**(1): e0190272.
50. World Health Organization. Global Malaria Programma: country antimalarial drug policies (Global AMDP database), 2018.
51. Frampton JE. Tafenoquine: First Global Approval. *Drugs* 2018; **78**(14): 1517-23.
52. GlaxoSmithKline. Krintafel (tafenoquine) tablets, for oral use: US prescribing information. 2018.
53. Rueangweerayut R, Bancone G, Harrell EJ, et al. Hemolytic Potential of Tafenoquine in Female Volunteers Heterozygous for Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency (G6PD Mahidol Variant) versus G6PD-Normal Volunteers. *Am J Trop Med Hyg* 2017; **97**(3): 702-11.
54. van der Pluijm RW, Imwong M, Chau NH, et al. Determinants of dihydroartemisinin-piperaquine treatment failure in Plasmodium falciparum malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. *Lancet Infect Dis* 2019; **19**(9): 952-61.
55. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in Plasmodium falciparum malaria. *N Engl J Med* 2009; **361**(5): 455-67.
56. Asua V, Conrad MD, Aydemir O, et al. Changing Prevalence of Potential Mediators of Aminoquinoline, Antifolate, and Artemisinin Resistance Across Uganda. *J Infect Dis* 2021; **223**(6): 985-94.
57. Uwimana A, Legrand E, Stokes BH, et al. Author Correction: Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. *Nat Med* 2021; **27**(6): 1113-5.
58. Nguyen TD, Gao B, Amaratunga C, et al. Preventing antimalarial drug resistance with triple artemisinin-based combination therapies. *Nat Commun* 2023; **14**(1): 4568.
59. Peto TJ, Tripura R, Callery JJ, et al. Triple therapy with artemether-lumefantrine plus amodiaquine versus artemether-lumefantrine alone for artemisinin-resistant, uncomplicated falciparum malaria: an open-label, randomised, multicentre trial. *Lancet Infect Dis* 2022; **22**(6): 867-78.

60. van der Pluijm RW, Tripura R, Hoglund RM, et al. Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated Plasmodium falciparum malaria: a multicentre, open-label, randomised clinical trial. *Lancet* 2020; **395**(10233): 1345-60.
61. Bousema T, Drakeley C. Epidemiology and infectivity of Plasmodium falciparum and Plasmodium vivax gametocytes in relation to malaria control and elimination. *Clin Microbiol Rev* 2011; **24**(2): 377-410.
62. Bradley J, Stone W, Da DF, et al. Predicting the likelihood and intensity of mosquito infection from sex specific Plasmodium falciparum gametocyte density. *eLife* 2018; **7**.
63. Lensen A, Bril A, van de Vegte M, van Gemert GJ, Eling W, Sauerwein R. Plasmodium falciparum: infectivity of cultured, synchronized gametocytes to mosquitoes. *Exp Parasitol* 1999; **91**(1): 101-3.
64. Barry A, Bradley J, Stone W, et al. Higher gametocyte production and mosquito infectivity in chronic compared to incident Plasmodium falciparum infections. *Nat Commun* 2021; **12**(1): 2443.
65. Lambrechts L, Halbert J, Durand P, Gouagna LC, Koella JC. Host genotype by parasite genotype interactions underlying the resistance of anopheline mosquitoes to Plasmodium falciparum. *Malar J* 2005; **4**: 3.
66. Mharakurwa S, Kumwenda T, Mkulama MA, et al. Malaria antifolate resistance with contrasting Plasmodium falciparum dihydrofolate reductase (DHFR) polymorphisms in humans and Anopheles mosquitoes. *Proc Natl Acad Sci U S A* 2011; **108**(46): 18796-801.
67. Gouagna LC, Bancone G, Yao F, et al. Genetic variation in human HBB is associated with Plasmodium falciparum transmission. *Nat Genet* 2010; **42**(4): 328-31.
68. Graves PM, Doubrovsky A, Sattabongkot J, Battistutta D. Human antibody responses to epitopes on the Plasmodium falciparum gametocyte antigen PFS 48/45 and their relationship to infectivity of gametocyte carriers. *Am J Trop Med Hyg* 1992; **46**(6): 711-9.
69. Graves PM, Carter R, Burkot TR, Quakyi IA, Kumar N. Antibodies to Plasmodium falciparum gamete surface antigens in Papua New Guinea sera. *Parasite Immunol* 1988; **10**(2): 209-18.
70. Bousema T, Sutherland CJ, Churcher TS, et al. Human immune responses that reduce the transmission of Plasmodium falciparum in African populations. *Int J Parasitol* 2011; **41** (3-4): 293-300.
71. Bousema T, Dinglasan RR, Morlais I, et al. Mosquito feeding assays to determine the infectiousness of naturally infected Plasmodium falciparum gametocyte carriers. *PLoS One* 2012; **7**(8): e42821.
72. de Jong RM, Meerstein-Kessel L, Da DF, et al. Monoclonal antibodies block transmission of genetically diverse Plasmodium falciparum strains to mosquitoes. *NPJ Vaccines* 2021; **6**(1): 101.
73. Ponnudurai T, Lensen AH, Van Gemert GJ, Bensink MP, Bolmer M, Meuwissen JH. Infectivity of cultured Plasmodium falciparum gametocytes to mosquitoes. *Parasitology* 1989; **98 Pt 2**: 165-73.
74. van der Kolk M, De Vlas SJ, Saul A, van de Vegte-Bolmer M, Eling WM, Sauerwein RW. Evaluation of the standard membrane feeding assay (SMFA) for the determination of malaria transmission-reducing activity using empirical data. *Parasitology* 2005; **130**(Pt 1): 13-22.
75. Sauerwein RW, Bousema T. Transmission blocking malaria vaccines: Assays and candidates in clinical development. *Vaccine* 2015; **33**(52): 7476-82.

76. Gerloff DL, Creasey A, Maslau S, Carter R. Structural models for the protein family characterized by gamete surface protein Pfs230 of Plasmodium falciparum. *Proc Natl Acad Sci U S A* 2005; **102**(38): 13598-603.
77. Eksi S, Czesny B, van Gemert GJ, Sauerwein RW, Eling W, Williamson KC. Malaria transmission-blocking antigen, Pfs230, mediates human red blood cell binding to exflagellating male parasites and oocyst production. *Mol Microbiol* 2006; **61**(4): 991-8.
78. van Dijk MR, Janse CJ, Thompson J, et al. A central role for P48/45 in malaria parasite male gamete fertility. *Cell* 2001; **104**(1): 153-64.
79. Stone WJR, Campo JJ, Ouedraogo AL, et al. Publisher Correction: Unravelling the immune signature of Plasmodium falciparum transmission-reducing immunity. *Nat Commun* 2018; **9**(1): 1498.
80. Vermeulen AN, Ponnudurai T, Beckers PJ, Verhave JP, Smits MA, Meuwissen JH. Sequential expression of antigens on sexual stages of Plasmodium falciparum accessible to transmission-blocking antibodies in the mosquito. *J Exp Med* 1985; **162**(5): 1460-76.
81. Rener J, Graves PM, Carter R, Williams JL, Burkot TR. Target antigens of transmission-blocking immunity on gametes of plasmodium falciparum. *J Exp Med* 1983; **158**(3): 976-81.
82. Barr PJ, Green KM, Gibson HL, Bathurst IC, Quakyi IA, Kaslow DC. Recombinant Pfs25 protein of Plasmodium falciparum elicits malaria transmission-blocking immunity in experimental animals. *J Exp Med* 1991; **174**(5): 1203-8.
83. Chaturvedi N, Bharti PK, Tiwari A, Singh N. Strategies & recent development of transmission-blocking vaccines against Plasmodium falciparum. *Indian J Med Res* 2016; **143**(6): 696-711.
84. Nikolaeva D, Draper SJ, Biswas S. Toward the development of effective transmission-blocking vaccines for malaria. *Expert Rev Vaccines* 2015; **14**(5): 653-80.
85. de Graaf H, Payne RO, Taylor I, et al. Safety and Immunogenicity of ChAd63/MVA Pfs25-IMX313 in a Phase I First-in-Human Trial. *Front Immunol* 2021; **12**: 694759.
86. Sagara I, Healy SA, Assadou MH, et al. Safety and immunogenicity of Pfs25H-EPA/Alhydrogel, a transmission-blocking vaccine against Plasmodium falciparum: a randomised, double-blind, comparator-controlled, dose-escalation study in healthy Malian adults. *Lancet Infect Dis* 2018; **18**(9): 969-82.
87. Talaat KR, Ellis RD, Hurd J, et al. Safety and Immunogenicity of Pfs25-EPA/Alhydrogel(R), a Transmission Blocking Vaccine against Plasmodium falciparum: An Open Label Study in Malaria Naive Adults. *PLoS One* 2016; **11**(10): e0163144.
88. Healy SA, Anderson C, Swihart BJ, et al. Pfs230 yields higher malaria transmission-blocking vaccine activity than Pfs25 in humans but not mice. *J Clin Invest* 2021; **131**(7).
89. Williamson KC, Keister DB, Muratova O, Kaslow DC. Recombinant Pfs230, a Plasmodium falciparum gametocyte protein, induces antisera that reduce the infectivity of Plasmodium falciparum to mosquitoes. *Mol Biochem Parasitol* 1995; **75**(1): 33-42.
90. Coelho CH, Tang WK, Burkhardt M, et al. Author Correction: A human monoclonal antibody blocks malaria transmission and defines a highly conserved neutralizing epitope on gametes. *Nat Commun* 2022; **13**(1): 4797.
91. Carter R, Graves PM, Keister DB, Quakyi IA. Properties of epitopes of Pfs 48/45, a target of transmission blocking monoclonal antibodies, on gametes of different isolates of Plasmodium falciparum. *Parasite Immunol* 1990; **12**(6): 587-603.

92. Roeffen W, Mulder B, Teelen K, et al. Association between anti-Pfs48/45 reactivity and P. falciparum transmission-blocking activity in sera from Cameroon. *Parasite Immunol* 1996; **18**(2): 103-9.

93. Theisen M, Jore MM, Sauerwein R. Towards clinical development of a Pfs48/45-based transmission blocking malaria vaccine. *Expert Rev Vaccines* 2017; **16**(4): 329-36.

94. Theisen M, Roeffen W, Singh SK, et al. A multi-stage malaria vaccine candidate targeting both transmission and asexual parasite life-cycle stages. *Vaccine* 2014; **32**(22): 2623-30.

95. Singh SK, Roeffen W, Andersen G, et al. A Plasmodium falciparum 48/45 single epitope R0.6C subunit protein elicits high levels of transmission blocking antibodies. *Vaccine* 2015; **33**(16): 1981-6.

96. Singh SK, Plieskatt J, Chourasia BK, et al. A Reproducible and Scalable Process for Manufacturing a Pfs48/45 Based Plasmodium falciparum Transmission-Blocking Vaccine. *Front Immunol* 2020; **11**: 606266.

97. Deng R, Wang Y, Maia M, et al. Pharmacokinetics and Exposure-Response Analysis of RG7667, a Combination of Two Anticytomegalovirus Monoclonal Antibodies, in a Phase 2a Randomized Trial To Prevent Cytomegalovirus Infection in High-Risk Kidney Transplant Recipients. *Antimicrob Agents Chemother* 2018; **62**(2).

98. Emu B, Fessel J, Schrader S, et al. Phase 3 Study of Ibalizumab for Multidrug-Resistant HIV-1. *N Engl J Med* 2018; **379**(7): 645-54.

99. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. The IMpact-RSV Study Group. *Pediatrics* 1998; **102**(3 Pt 1): 531-7.

100. Lyke KE, Berry AA, Mason K, et al. Low-dose intravenous and subcutaneous CIS43LS monoclonal antibody for protection against malaria (VRC 612 Part C): a phase 1, adaptive trial. *Lancet Infect Dis* 2023; **23**(5): 578-88.

101. Roeffen W, Teelen K, van As J, vd Vegte-Bolmer M, Eling W, Sauerwein R. Plasmodium falciparum: production and characterization of rat monoclonal antibodies specific for the sexual-stage Pfs48/45 antigen. *Exp Parasitol* 2001; **97**(1): 45-9.

102. Kundu P, Semesi A, Jore MM, et al. Structural delineation of potent transmission-blocking epitope I on malaria antigen Pfs48/45. *Nat Commun* 2018; **9**(1): 4458.

103. World Health Organization. World malaria report 2023. Geneva, 2023.

104. Rogers DJ, Randolph SE. Climate change and vector-borne diseases. *Adv Parasitol* 2006; **62**: 345-81.

105. Nissan H, Ukawuba I, Thomson M. Correction to: Climate-proofing a malaria eradication strategy. *Malar J* 2021; **20**(1): 215.

106. Campbell-Lendrum D, Manga L, Bagayoko M, Sommerfeld J. Climate change and vector-borne diseases: what are the implications for public health research and policy? *Philos Trans R Soc Lond B Biol Sci* 2015; **370**(1665).

107. World Health Organization. Malaria eradication: benefits, future scenarios and feasibility: a report of the Strategic Advisory Group on Malaria Eradication. . Geneva, 2020.

108. Craig MH, Snow RW, le Sueur D. A climate-based distribution model of malaria transmission in sub-Saharan Africa. *Parasitol Today* 1999; **15**(3): 105-11.

109. Dye C, Reiter P. Climate change and malaria: temperatures without fevers? *Science (80-)* 2000; **289**(5485): 1697-8.

110. Weiss DJ, Bhatt S, Mappin B, et al. Air temperature suitability for Plasmodium falciparum malaria transmission in Africa 2000-2012: a high-resolution spatiotemporal prediction. *Malar J* 2014; **13**: 171.

111. Guerra CA, Gikandi PW, Tatem AJ, et al. The limits and intensity of Plasmodium falciparum transmission: implications for malaria control and elimination worldwide. *PLoS Med* 2008; **5**(2): e38.

112. Gething PS, T.; Woods, W.; Sukitsch, N.; Potere, D.; Desai, N.; Balzarolo, A.; Moran, A. Climate impacts on malaria in Africa: The Kids Research Institute Australia, Curtin University and Boston Consulting Group, 2024.

113. Romanello M, Di Napoli C, Drummond P, et al. The 2022 report of the Lancet Countdown on health and climate change: health at the mercy of fossil fuels. *Lancet* 2022; **400**(10363): 1619-54.

114. De Vincenzo R, Conte C, Ricci C, Scambia G, Capelli G. Long-term efficacy and safety of human papillomavirus vaccination. *Int J Womens Health* 2014; **6**: 999-1010.

115. Thrane S, Janitzek CM, Agerbaek MO, et al. A Novel Virus-Like Particle Based Vaccine Platform Displaying the Placental Malaria Antigen VAR2CSA. *PLoS One* 2015; **10**(11): e0143071.

Part 1: Unveiling the *Plasmodium falciparum* transmission-reducing potential of antimalarials

CHAPTER 2

Single low dose tafenoquine combined with dihydroartemisinin–piperaquine to reduce *Plasmodium falciparum* transmission in Ouélessébougou, Mali: a phase 2, single-blind, randomised clinical trial

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Summary

Background

Tafenoquine was recently approved as a prophylaxis and radical cure for *Plasmodium vivax* infection, but its *Plasmodium falciparum* transmission-blocking efficacy is unclear. We aimed to establish the efficacy and safety of three single low doses of tafenoquine in combination with dihydroartemisinin–piperaquine for reducing gametocyte density and transmission to mosquitoes.

Methods

In this four-arm, single-blind, phase 2, randomised controlled trial, participants were recruited at the Clinical Research Unit of the Malaria Research and Training Centre of the University of Bamako in Mali. Eligible participants were aged 12–50 years, with asymptomatic *P falciparum* microscopy-detected gametocyte carriage, had a bodyweight of 80 kg or less, and had no clinical signs of malaria defined by fever. Participants were randomly assigned (1:1:1:1) to standard treatment with dihydroartemisinin–piperaquine, or dihydroartemisinin–piperaquine plus a single dose of tafenoquine (in solution) at a final dosage of 0·42 mg/kg, 0·83 mg/kg, or 1·66 mg/kg. Randomisation was done with a computer-generated randomisation list and concealed with sealed, opaque envelopes. Dihydroartemisinin–piperaquine was administered as oral tablets over 3 days (day 0, 1, and 2), as per manufacturer instructions. A single dose of tafenoquine was administered as oral solution on day 0 in parallel with the first dose of dihydroartemisinin–piperaquine. Tafenoquine dosing was based on bodyweight to standardise efficacy and risk variance. The primary endpoint, assessed in the per-protocol population, was median percentage change in mosquito infection rate 7 days after treatment compared with baseline. Safety endpoints included frequency and incidence of adverse events. The final follow-up visit was on Dec 23, 2020; the trial is registered with ClinicalTrials.gov, NCT04609098.

Findings

From Oct 29 to Nov 25, 2020, 1091 individuals were screened for eligibility, 80 of whom were enrolled and randomly assigned (20 per treatment group). Before treatment, 53 (66%) individuals were infectious to mosquitoes, infecting median 12·50% of mosquitoes (IQR 3·64–35·00). Within-group reduction in mosquito infection rate on day 7 was 79·95% (IQR 57·15–100; p=0·0005 for difference from baseline) following dihydroartemisinin–piperaquine only, 100% (98·36–100; p=0·0005) following dihydroartemisinin–

piperaquine plus tafenoquine 0·42 mg/kg, 100% (100–100; p=0·0001) following dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg, and 100% (100–100; p=0·0001) following dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg. 55 (69%) of 80 participants had a total of 94 adverse events over the course of the trial; 86 (92%) adverse events were categorised as mild, seven (7%) as moderate, and one (1%) as severe. The most common treatment-related adverse event was mild or moderate headache, which occurred in 15 (19%) participants (dihydroartemisinin–piperaquine n=2; dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg n=6; dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg n=3; and dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg n=4). No serious adverse events occurred. No significant differences in the incidence of all adverse events (p=0·73) or treatment-related adverse events (p=0·62) were observed between treatment groups.

Interpretation

Tafenoquine was well tolerated at all doses and accelerated *P falciparum* gametocyte clearance. All tafenoquine doses showed improved transmission reduction at day 7 compared with dihydroartemisinin–piperaquine alone. These data support the case for further research on tafenoquine as a transmission-blocking supplement to standard antimalarials.

Research in context

Evidence before this study

Primaquine and its long-acting analogue, tafenoquine, belong to the class of 8-aminoquinolines that can clear *Plasmodium vivax* liver stages and thereby form an essential component of *P vivax* radical cure. Primaquine is also a potent *Plasmodium falciparum* gametocytocide; a single low dose of primaquine combined with standard artemisinin-combination therapy (ACT) prevents transmission by rapidly sterilising and killing *P falciparum* gametocytes. The potency of tafenoquine as a *P falciparum* gametocytocide has so far remained unstudied. We searched PubMed on June 22, 2021, with no restrictions on publication date or language, for studies assessing the transmission-blocking and gametocytocidal abilities of tafenoquine treatment with search terms: ([Tafenoquine] OR [Krintafel] OR [Arakoda] OR [WR-238605]) AND ([Gametocytocidal] OR [Gametocytes] OR [Transmission]). Among the

16 results (1992–2019) meeting the search criteria, none of the identified studies assessed the *P falciparum* gametocytocidal and transmission-blocking properties of tafenoquine in naturally infected gametocyte carriers. The results consisted of the following: four reviews; two studies assessing the sporontocidal activity of tafenoquine against *P vivax*; one study assessing glucose-6-phosphate dehydrogenase deficiency prevalence in Ethiopia; one study comparing in-vitro sensitivity of *P falciparum* isolates against three 8-aminoquinolines; one study investigating drug interactions between tafenoquine and ACTs; two articles regarding the development of tafenoquine; one study in which the prophylactic efficacy of tafenoquine was studied in a human *P falciparum* challenge model; and four studies wherein gametocytocidal and sporontocidal activity of tafenoquine was tested in avian and rodent models.

Added value of this study

Transmission-blocking antimalarials are important components of strategies to eliminate malaria and prevent the spread of drug-resistant *Plasmodium*. The long-acting 8-aminoquinoline tafenoquine might have unique properties in blocking the transmission of malaria over a prolonged period and possibly multiple infections. To our knowledge, this is the first clinical trial assessing the *P falciparum* gametocytocidal and transmission-blocking properties of single low doses of tafenoquine. Three different single low doses of tafenoquine were combined with dihydroartemisinin–piperaquine. The recommended dose of tafenoquine for radical cure of *P vivax* is 300 mg (ie, 5 mg/kg in an adult weighing 60 kg). The maximum dose used in the current study was one third of this recommended dose (1·66 mg/kg, equivalent to 100 mg total dose in an adult weighing 60 kg). The results show that tafenoquine accelerated *P falciparum* gametocyte clearance in a dose-dependent manner and greatly reduced transmission. Although findings from previous studies suggest that primaquine's efficacy is evident within the first 48 h after administration, tafenoquine's transmission-blocking occurred later; first detected on day 7 after initiation of treatment. These data support the case for further research on tafenoquine as a transmission-blocking supplement to standard antimalarials.

Implications of all the available evidence

This study provides the first evidence that tafenoquine is gametocytocidal in humans and prevents transmission to mosquitoes by day 7 after

treatment. Some individuals treated with dihydroartemisinin–piperaquine alone were still infectious to mosquitoes up to the last day of mosquito feeding assays (day 14 after treatment), corroborating earlier reports on the persistence of transmissible gametocytes after artemisinin-combination therapy. By contrast, none of the individuals who received tafenoquine were infectious by day 14 and the majority of transmission events were prevented by day 7. The current findings highlight the importance of gametocytocidal and transmission-blocking supplements in areas trying to reduce the malaria infectious reservoir and identify tafenoquine as a new *P falciparum* gametocytocide.

Introduction

After years of success in malaria control, efforts to further reduce the global incidence of malaria are stalled. There were an estimated 229 million cases of malaria worldwide in 2019, up from 217 million in 2014.[1] With spreading resistance to first-line drugs against *Plasmodium falciparum* malaria, there is a clear need for interventions focused on reducing malaria parasite transmission.

Artemisinin combination therapies (ACTs) clear *P falciparum* asexual parasites but have little activity against mature gametocytes. Gametocytes are the only *Plasmodium* life stage that can be transmitted to mosquitoes, so their prevalence, density, and distribution within the human population define the malaria infectious reservoir. In areas consolidating malaria elimination or aiming to contain the spread of artemisinin resistance, WHO recommends that ACTs be combined with a single low dose of the 8-aminoquinoline compound, primaquine.[2] Single low-dose primaquine combined with ACT prevents transmission by rapidly sterilising and killing gametocytes, but its short half-life (4–9 h) might prevent primaquine from affecting any gametocytes that arise late during infections or develop after recrudescence. The effect of single low-dose primaquine might therefore be limited to the sterilisation of currently circulating gametocytes.[3] Another 8-aminoquinoline, tafenoquine, has recently been approved as a single-dose radical cure for *Plasmodium vivax*[4] and its potential as a *P falciparum* gametocytocide is suggested by in-vitro[5] and murine models.[6] The long half-life (15 days)[7] of tafenoquine could be a major advantage over short-lived gametocytocidal treatments, but comes with a risk of prolonged haemolysis for individuals deficient in the production of glucose-6-phosphate dehydrogenase (G6PD),[8] who can have acute haemolytic

anaemia after radical curative treatment of *P vivax* malaria with primaquine. Recent trials indicate that a single dose of 300 mg tafenoquine is considered safe among individuals with normal G6PD production and has equivalent transient haemolytic activity to standard 14-day primaquine regimens for *P vivax* treatment in women who are deficient in G6PD.[8]

Tafenoquine has not yet been tested in controlled trials for its ability to clear and prevent transmission of *P falciparum* gametocytes. Dosing for a gametocytocidal indication has not been assessed but is likely to be substantially lower than the required dose for radical cure of *P vivax* (as is the case with primaquine). In the current study, we assessed the gametocytocidal and transmission-blocking efficacy of a range of single low doses of tafenoquine (0·41 mg/kg, 0·83 mg/kg, and 1·66 mg/kg) in combination with dihydroartemisinin–piperaquine in Malian children and adults with normal G6PD production.

Methods

Study design and participants

This four-arm, single-blind, phase 2, randomised controlled trial was conducted at the Clinical Research Unit of the Malaria Research and Training Centre (MRTC) of the University of Bamako in Mali. Before the commencement of screening, our study team of clinicians and technicians met with community leaders, village health workers, and heads of households from each village to explain the study and obtain approval to conduct the study. Village health workers then used a door-to-door approach to inform households of the date and location where consenting and screening would take place. Participants were included in the trial if they met the following criteria: positive for *P falciparum* gametocytes by microscopy (ie, ≥1 gametocytes recorded in a thick film against 500 white blood cells, equating to 16 gametocytes per µL with a standard conversion of 8000 white blood cells per µL blood); absence of other non-*P falciparum* species on blood film; haemoglobin density of 10 g/dL or more; normal G6PD (male >4 IU/g haemoglobin, female >6 IU/g haemoglobin); aged between 12–50 years; bodyweight 80 kg or less; no clinical signs of malaria defined by fever (≥37·5°C); no signs of acute, severe, or chronic disease; no allergies to any study drugs; reported no use of antimalarial drugs over the past week; consistent with the long half-life of tafenoquine, use of effective contraception for five half-lives (3 months) after the end

of tafenoquine treatment. Exclusion criteria included pregnancy (tested at enrolment by urine and serum test) or lactation, use of other medication (except for paracetamol or aspirin), family history of congenital prolongation of the corrected QT interval, current or recent treatment with drugs known to extend the corrected QT interval, and blood transfusion in the past 90 days. Before screening and before study enrolment, participants provided written informed consent (aged ≥18 years) or assent with written parental consent (aged 12–17 years).

Ethical approval was granted by the Ethics Committee of the Faculty of Medicine, Pharmacy, and Dentistry of the University of Science, Techniques, and Technologies of Bamako (Bamako, Mali), and the Research Ethics Committee of the London School of Hygiene & Tropical Medicine (London, UK).

Randomisation and masking

Participants were randomly assigned (1:1:1:1) to receive dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg, dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg, dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg, or dihydroartemisinin–piperaquine alone. Enrolment continued until 80 participants were enrolled (20 individuals assigned to each treatment group). An independent MRTC statistician randomly generated the treatment assignment using Stata (version 16), which was linked to participant identification number. The statistician prepared sealed, opaque envelopes with the participant identification number on the outside and treatment assignment inside, which were sent to the MRTC study pharmacist. The study pharmacist provided treatment according to the contained assignment and was consequently not masked to treatment assignment. All other investigators and staff involved in assessing all laboratory outcomes were masked. Participants could ask the study physician which treatment they received.

Procedures

Dihydroartemisinin–piperaquine was administered as oral tablets over 3 days (day 0, 1, and 2), as per manufacturer instructions (see appendix 5 p 2 for dosing). A single dose of tafenoquine was administered as oral solution on day 0 in parallel with the first dose of dihydroartemisinin–piperaquine. Tafenoquine dosing was based on bodyweight to standardise efficacy and risk variance (supplementary information 1). The maximum dose chosen for the current study was based on equivalent safety profile of tafenoquine doses of 300 mg or less and to standard primaquine dosing (15 mg daily for 14 days)

in G6PD heterozygous adults (full details in supplementary information 1).[8] The recommended dose of tafenoquine for radical cure of *P vivax* is 300 mg (ie, 5 mg/kg in an adult weighing 60 kg).[7] The maximum dose used in the current study was 1·66 mg/kg, equivalent to 100 mg total dose in an adult weighing 60 kg. G6PD testing was done using both semiquantitative (OSMMR-D G-6-PD Test; R&D Diagnostics, Aghia Paraskevi, Greece) and quantitative testing (STANDARD G6PD Test; SD Biosensor, Suwon, South Korea); inclusion in the study required normal enzyme function to be determined by both methods. The thresholds used for normal G6PD activity were more than 30% for male participants and 70% for female participants.

Participants received a full clinical and parasitological examination on days 1, 2, 7, 14, 21, and 28 after receiving the first dose of the study drugs. Giemsa stained thick film microscopy was performed as described previously, with asexual stages counted against 200 white blood cells and gametocytes counted against 500 white blood cells.[9] For molecular gametocyte quantification, total nucleic acids were extracted using a MagNAPure LC automated extractor (Total Nucleic Acid Isolation Kit-High Performance; Roche Applied Science, Indianapolis, IN, USA). Male and female gametocytes were quantified in a multiplex reverse transcriptase quantitative PCR (RT-qPCR) assay,[10] targeting CCP4/PfMGET mRNA (supplementary table 1). Samples were classified as negative for a particular gametocyte sex if the RT-qPCR quantified density of gametocytes of that sex was less than 0·01 gametocytes per µL (ie, one gametocyte per 100 µL of blood sample). If accurate gametocyte quantification was not possible (eg, due to uncertainty over starting blood volume), density values were removed from analysis while prevalence values were retained. Haemoglobin density was measured using a haemoglobin analyser (HemoCue; AB Leo Diagnostics, Helsingborg, Sweden) or automatic haematology analyser (HumaCount 5D; Human Diagnostics Worldwide, Wiesbaden, Germany). Methaemoglobin was measured non-invasively using a Masimo Rainbow SET platform (Masimo Corporation, Irvine, CA, USA). Methaemoglobin saturation measures of 0% were confirmed as methodological errors and removed from analysis. Additional venous blood samples were taken for biochemical and infectivity assessments on days 0, 2, 7, and 14 in all treatment groups. Aspartate aminotransferase, alanine aminotransferase, and blood creatine concentrations were measured using automatic biochemistry analyser HumaStar 100 (Human Diagnostics Worldwide, Wiesbaden, Germany). For each assessment of infectivity, approximately 75 locally reared *Anopheles gambiae* were allowed to feed for 15–20 min on venous blood samples (Lithium

Heparin VACUETTE tube; Greiner Bio-One, Kremsmünster, Austria) through a prewarmed glass membrane feeder system (Coelen Glastechniek, Weldaad, Netherlands). All surviving mosquitoes were dissected on the seventh day after the feeding assay; midguts were stained in 1% mercurochrome and examined for the presence and density of oocysts by expert microscopists.

Outcomes

The primary outcome measure was median percentage change in mosquito infection rate between pretreatment and 7 days after treatment. Secondary outcomes were mosquito infection metrics (oocyst density, mosquito infection rate, and infectious individuals) at other prespecified timepoints (day 0, 2, 7, and 14); gametocyte prevalence, density, circulation time, area under the curve (AUC) of density over time, and sex ratio (ie, proportion of gametocytes that were male); and safety assessments including incidence of clinical and laboratory adverse events. Differences in all transmission, gametocyte, and safety outcomes were compared between treatment groups (individual tafenoquine groups compared with the reference dihydroartemisinin–piperaquine-only treatment group) as secondary outcomes.

Adverse events were graded by the study clinician for severity (mild, moderate, or severe) and relatedness to study medication (unrelated or unlikely, possibly, probably, or definitely related). A reduction in haemoglobin concentration of 40% or more from baseline was categorised as a haematological adverse event. An external data safety and monitoring committee was assembled before the trial, and safety data were discussed after enrolment of 40 participants, and after the final follow-up visit of the last participant.

Statistical analysis

Sample size estimation was based on efficacy for single-dose primaquine (in the absence of infectivity level data on tafenoquine, and assuming equivalence) to provide a 95% or greater reduction in infectivity at 7 days after initiation of treatment compared with pretreatment using a membrane feeding assay.[11] With 20 participants per group, we would have 80% power to detect a 95% or greater reduction in the number of mosquitoes with oocysts after treatment as significant at the 0·05 level. The sample size was designed to assess change in infectivity within treatment groups, not compare transmission reducing effects between treatment groups.

All outcomes were analysed in the per-protocol population. Mosquito infectivity was assessed at three levels: mean number of oocysts in a sample of mosquitoes (ie, oocyst density), the proportion of mosquitoes infected with any number of oocysts (ie, mosquito infection rate), and infectivity of the study participant to any number of mosquitoes (ie, infectious individuals). Asexual parasite density was not measured by molecular methods due to the scarcity of nucleic acid extraction reagents.

Mosquito infection rate and oocyst density were analysed at timepoints after baseline only for those individuals who were infectious at baseline. The prevalence of gametocytes and infectious individuals were compared within and between treatment groups using one-sided Fishers exact tests. Haemoglobin and methaemoglobin levels were compared using paired *t* tests (*t* score) for within-group analyses and linear regression adjusted for baseline levels of each measure for between-group analyses (*t* score, coefficient with 95% CI). Percentage change from baseline was analysed using two-way *t* tests. The proportion of gametocytes that were male was analysed for all values with total gametocyte densities of 0·2 gametocytes per µL or more.[9] Gametocyte circulation time was calculated to determine the mean number of days that a mature gametocyte circulates in the blood before clearance, using a deterministic compartmental model that assumes a constant rate of clearance and has a random effect to account for repeated measures on individuals, as described previously;[12] difference in circulation time between groups and between gametocyte sexes was analysed using *t* tests (*t* score), and the dose effect of tafenoquine on circulation time was determined using linear regression analysis. AUC of gametocyte density per participant over time was calculated using the linear trapezoid method,[13] and was analysed by fitting linear regression models to the $\log_{10}$ adjusted AUC values, with adjustment for baseline gametocyte density (*t* score, coefficient with 95% CI). All other analyses of quantitative data were done using Wilcoxon sign rank tests (z-score) and Wilcoxon rank-sum tests (z-score). All comparisons were defined before study completion and analyses were not adjusted for multiple comparisons. For all analyses, the threshold for statistical significance was set at $p<0·05$. Statistical analysis was conducted using STATA (version 16.0) and SAS (version 9.4). The trial is registered with ClinicalTrials.gov, NCT04609098.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Oct 29 and Nov 25, 2020, 1091 individuals were screened for eligibility, 80 of whom were enrolled and randomly assigned (20 to each treatment group; figure 1). Participant baseline characteristics were similar between the study groups, although the proportion of male participants was higher in the dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg and 1·66 mg/kg groups than in the dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg and dihydroartemisinin–piperaquine-only groups (table 1).

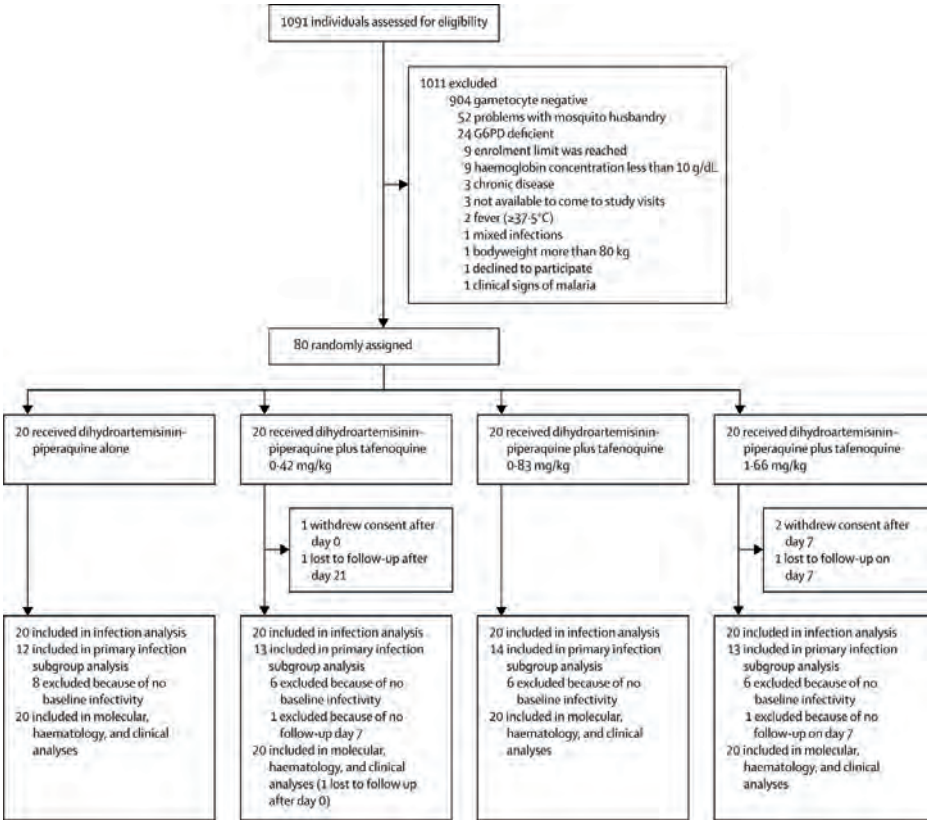

Figure 1. Trial profile. G6PD=glucose-6-phosphate dehydrogenase.

	Dihydroartemisinin–piperaquine (n=20)	Dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg (n=20)	Dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg (n=20)	Dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg (n=20)
Age, years	17 (14–19)	13 (12–17)	15 (12–32)	15 (13–21)
Female	8 (40%)	8 (40%)	6 (30%)	6 (30%)
Male	12 (60%)	12 (60%)	14 (70%)	14 (70%)
Haemoglobin, g/dL	12·6 (10·9–15·7)	12·3 (10·2–15·8)	12·4 (11·1–14·8)	12·9 (10·8–15·0)
Gametocyte prevalence	20 (100%)	20 (100%)	20 (100%)	20 (100%)
Gametocyte density, parasites per µL	41·5 (12·5–95·9)	29·5 (12·3–67·8)	43·7 (18·5–245·0)	50·1 (17·8–201·8)
Asexual parasite prevalence	12 (60%)	11 (55%)	8 (40%)	15 (75%)
Asexual parasite density, parasites per µL	120 (0–560)	60 (0–300)	0 (0–80)	500 (60–1840)

Table 1. Baseline characteristics. Data are median (IQR), n (%), or median (IQR). Gametocyte prevalence and density were calculated from reverse transcriptase quantitative PCR targeting CCP4/PfMGET mRNA (gametocytes). Asexual parasite prevalence and density were assessed by thick film microscopy.

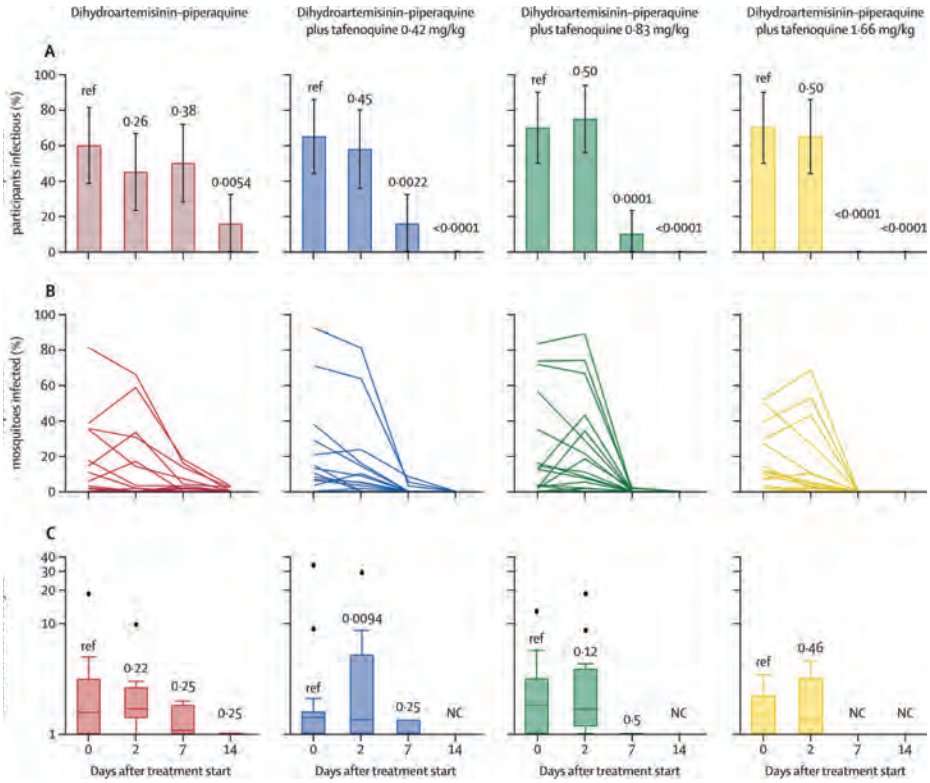

Figure 2. Participant infectivity, proportion of mosquitoes infected, and oocyst density in direct membrane feeding assays. (A) Participant infectivity. Error bars are 95% CIs. p values from generalised linear models (family: binary) testing differences within treatment groups with baseline as reference are shown. The denominator for the proportion of infectious participants is the total number of participants still enrolled at a given timepoint, rather than the number tested at that time point for infectivity; infectivity assays were discontinued when a participant did not infect mosquitoes at two subsequent timepoints and were thereafter considered non-infectious. Full mosquito infection data including the proportion of infectious participants with denominator as total participants tested is shown in supplementary table 2). (B) Mosquito infection rate. Each line represents one participant. Statistical analyses are shown in supplementary table 2). (C) Oocyst density. Box plots show the median (central line), IQR (box limits), upper and lower quartiles plus 1·5 × IQR (whiskers), and outliers for mean oocyst densities in infected mosquitoes within each participant. Wilcoxon sign rank tests for differences in average oocyst density are shown. NC=not calculable.

The primary outcome measure was recorded on day 7 of follow-up, with 79 (99%) of 80 individuals completing this study visit (one in the dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg group did not complete the visit). 76 (95%) participants completed all visits to day 28 (two in the dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg group and two in the dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg group did not complete all visits). The median number of mosquitoes dissected in an individual mosquito feeding experiment was 55 (IQR 45–62). Before treatment, 53 (66%) individuals were infectious to mosquitoes, with a median of 12·50% (IQR 3·64–35·00) of mosquitoes becoming infected (table 2). The proportion of individuals who infected any mosquitoes at day 7 is shown in table 2 (figure 2; supplementary table 2). At day 7 there was a significant within-person reduction in mosquito infection rate in all four treatment groups (relative to baseline in each group), although the median reduction was greater in the three groups with tafenoquine than in the dihydroartemisinin–piperaquine-only group (table 2). At day 2, the proportion of individuals who were infectious to mosquitoes was not significantly different from baseline (figure 2; supplementary table 2) and the reduction in mosquito infection rate was not significant for any treatment group (table 2). The final mosquito feeds were done at day 14 after treatment, at which point three (16%) of 19 individuals in the dihydroartemisinin–piperaquine-only group were still infectious to mosquitoes, with a median mosquito infection rate of 0% (IQR 0–1·06) among individuals positive at baseline, and 2·38% (2·13–3·03) among those positive at day 14; no participants were infectious at day 14 in the tafenoquine treatment groups. Oocyst densities in infected mosquitoes were not significantly different between treatment groups at any pretreatment or post-treatment timepoint (figure 2; supplementary table 2). There were also no significant differences in oocyst density relative to baseline until day 7 post-treatment, at which point oocyst densities were significantly reduced in all groups (figure 2; supplementary table 2).

Gametocyte densities decreased after initiation of treatment in all study groups, although the decrease was more rapid in the tafenoquine groups than in the dihydroartemisinin–piperaquine-only group (figure 3; supplementary table 4A). Total gametocyte prevalence by the final day of follow-up (day 28) was 18 (90%) of 20 participants in the dihydroartemisinin–piperaquine-only group, 12 (67%) of 18 participants in the dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg group, 13 (65%) of 20 participants in the dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg group, and

Table 2. Infectivity to mosquitoes before and after treatment.

	Infectious individuals*	Median mosquito infection rate† (IQR)	Median reduction in mosquito infection rate‡ (IQR)	p value§	p value¶
Pretreatment					
Dihydroartemisinin–piperaquine	12/20 (60%)	12·50% (2·44 to 35·24)	..	..	..
Dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg	13/20 (65%)	12·96% (6·67 to 28·79)	..	..	..
Dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg	14/20 (70%)	13·39% (3·03 to 56·52)	..	..	..
Dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg	14/20 (70%)	11·35% (6·82 to 29·17)	..	..	..
Day 2					
Dihydroartemisinin–piperaquine	9/20 (45%)	8·63% (0 to 31·92)	70·68% (–19·34 to 100)	0·33	Ref
Dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg	11/19 (58%)	9·39% (4·09 to 19·71)	36·17% (11·33 to 54·25)	0·063	0·50
Dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg	15/20 (75%)	19·77% (7·35 to 43·08)	15·35% (–100 to 48·96)	1·00	0·094
Dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg	13/20 (65%)	7·59% (2·38 to 26·79)	51·23% (–36·67 to 68·63)	0·12	0·58
Day 7					
Dihydroartemisinin–piperaquine	10/20 (50%)	2·36% (0 to 10·39)	79·95% (57·15 to 100)	0·0005	Ref
Dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg	3/19 (16%)	0% (0 to 1·52)	100% (98·36 to 100)	0·0005	0·020
Dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg	2/20 (10%)	0% (0 to 0)	100% (100 to 100)	0·0001	0·011

	Infectious individuals*	Median mosquito infection rate† (IQR)	Median reduction in mosquito infection rate‡ (IQR)	p value§	p value¶
Dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg	0/19	0% (0 to 0)	100% (100 to 100)	0·0001	0·0006
Day 14					
Dihydroartemisinin–piperaquine	3/19 (16%)	0% (0 to 1·06)	0% (0 to 1·06)	0·0005	Ref
Dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg	0/19	0% (0 to 0)	0% (0 to 0)	0·0005	0·22
Dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg	0/19	0% (0 to 0)	0% (0 to 0)	0·0001	0·17
Dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg	0/17	0% (0 to 0)	0% (0 to 0)	0·0005	0·22

Table 2. Infectivity to mosquitoes before and after treatment.

* Individuals were classed as infectious if direct membrane feeding assays resulted in at least one mosquito with any number of oocysts. Mosquito infection measures (percentage infection and oocyst density) are shown for all participants who were infectious at baseline, and oocyst densities are from all infected mosquitoes.

† Median proportion of mosquitoes infected by each participant, where for each participant the mosquito infection rate was the number of mosquitoes infected as a proportion of all mosquitoes surviving to dissection.

‡ Median reduction (relative to baseline) in mosquito infection rate at the given timepoints. All values are for individuals who were infectious to mosquitoes before treatment (ie, infected any number of mosquitoes).

§ Within-group comparison in median reduction in mosquito infection rate (primary outcome).

¶ Between-group comparison in median reduction in mosquito infection rate. Full details of mosquito feeding assay outcomes are in supplementary table 2 and 3.

three (17%) of 18 in the dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg group. Total gametocyte circulation time was estimated at 8·3 days (95% CI 7·0–9·6) in the dihydroartemisinin–piperaquine-only group, and decreased to 2·7 days (2·5–2·9) in the dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg group. We observed evidence for a dose–response effect of tafenoquine on gametocyte clearance; doubling the tafenoquine dose was associated with a decrease in circulation time of 1·2 days (0·9–1·5) for female gametocytes and 0·5 days (0·3–0·7) for male gametocytes. Gametocyte AUC was similarly lower in the tafenoquine treatment groups than in the dihydroartemisinin–piperaquine-only group, after adjustment for baseline gametocyte density (supplementary table 3). Gametocyte sex ratios were initially similar in all treatment groups (median proportion male 0·51 [IQR 0·42–0·62]) but female gametocyte density decreased substantially in the dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg group by day 7 after treatment, resulting in significantly male-biased sex ratios (supplementary table 4A and supplementary figure 2). From day 14, total gametocyte densities became too low to reliably determine sex ratio in the majority of samples in the dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg group (eight samples remained with densities >0·2 per µL). At day 7, the infectivity of persisting gametocytes was significantly lower in the tafenoquine treatment groups compared with the dihydroartemisinin treatment group (supplementary table 4B).

There was a small but statistically significant within-group reduction in haemoglobin density in the dihydroartemisinin–piperaquine-only and dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg groups at days 2 and 7 compared with baseline, with no significant reductions in any other treatment group or at any other timepoint (supplementary figure 2 and 3). The greatest mean reduction in haemoglobin density in any treatment group or timepoint was −3·3% (95% CI −4·7 to −1·8) in the dihydroartemisinin–piperaquine-only group at day 7 (supplementary figure 2). The greatest reduction in haemoglobin density in any individual was 25·6% (from 13·3 g/dL at baseline to 9·9 g/dL at day 21 in an individual in the dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg group). This was the lowest observed haemoglobin density in any individual and timepoint and increased to 10·3 g/dL at the next visit. Methaemoglobin concentration was significantly increased from baseline between days 7 and 21 in the dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg and 1·66 mg/kg groups, but was only significantly greater than the dihydroartemisinin–piperaquine-only group in the dihydroartemisinin–

piperaquine plus tafenoquine 1·66 mg/kg group (at all timepoints other than baseline and day 21; supplementary table 7 and supplementary figure 3).

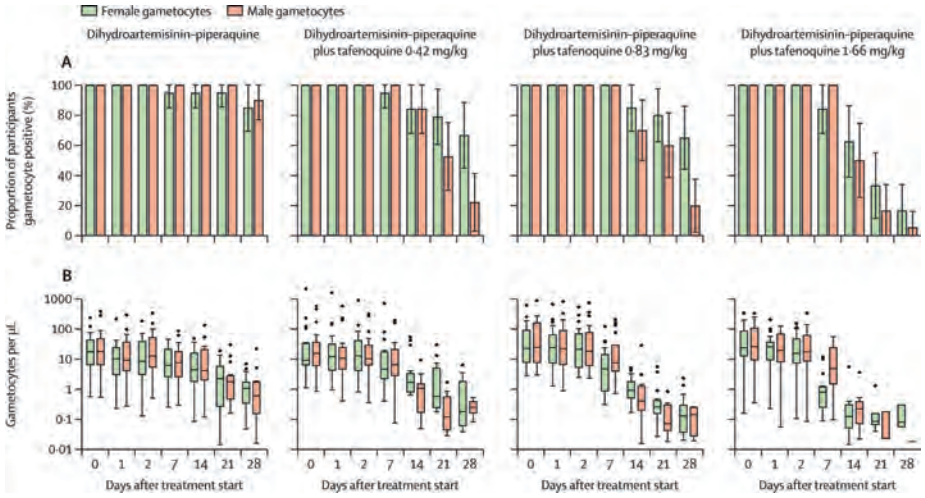

Figure 3. Male and female gametocyte density and prevalence. (A) Gametocyte prevalence. Error bars are 95% CIs. (B) Gametocyte density, shown for gametocyte positive individuals only (ie, male or female density >0·01 per µL). Box plots show the median (central line), IQR (box limits), upper and lower quartiles plus 1·5 × IQR (whiskers), and outliers. Within and between group statistical analyses of gametocyte density and prevalence are shown in supplementary table 4A). Gametocyte circulation time, area under the curve, and other outcomes are shown in supplementary table 3, 4A, 4B, 5, 6 and supplementary figure 2).

Overall, 55 (69%) of 80 participants had a total of 94 adverse events during follow-up, of which 86 (92%) were categorised as mild, seven (7%) as moderate, and one (1%) as severe (supplementary table 8 and 9). The single severe adverse event was a urinary tract infection not related to the study in a 25-year-old woman in the dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg group at day 7. This infection was resolved without sequela with appropriate treatment. There was no difference between treatment groups in the proportion of participants who had any adverse event (p=0·73), or adverse events of mild (p=0·95) or moderate (p=0·75) severity. The most common treatment-related adverse event was mild or moderate headache, which occurred in 15 (19%) participants (dihydroartemisinin–piperaquine n=2; dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg n=6; dihydroartemisinin–piperaquine plus tafenoquine 0·83 mg/kg n=3; and dihydroartemisinin–piperaquine plus tafenoquine 1·66 mg/kg n=4). No serious adverse events occurred. There was no difference between treatment groups in the proportion of participants who had any adverse event that was possibly, probably, or definitely

related to treatment (p=0·62; supplementary table 8). No individuals had clinically significant creatinine, aspartate aminotransferase, or alanine aminotransferase concentrations outside the normal range after treatment. In the dihydroartemisinin–piperaquine plus tafenoquine 0·42 mg/kg group, two individuals had transient increases in alanine aminotransferase outside the normal range, categorised as mild adverse events, on day 2 and 7, and one individual had a transient increase in creatine at day 7, categorised as a moderate adverse event. All were classified as possibly drug related, and all normalised on the subsequent visit (supplementary table 10).

Discussion

To our knowledge, this was the first clinical trial specifically designed to determine the *P falciparum* gametocytocidal and transmission-blocking properties of single low doses of tafenoquine. By day 7, post-treatment transmission potential was greatly reduced in the 0·42 and 0·83 mg/kg tafenoquine dose groups, and completely annulled in individuals given the highest (1·66 mg/kg) tafenoquine dose.

ACTs differ in their gametocytocidal properties.[14] Gametocyte clearance by antimalarial drugs might be preceded by distortions in gametocyte sex ratio or gametocyte fitness that prevent onward transmission to mosquitoes,[15,16] highlighting the value of true assessment of infectivity through mosquito feeding assays when examining a drug's transmission-reducing properties. The findings from our study support earlier reports of persisting gametocyte carriage and transmission in the weeks following treatment with dihydroartemisinin–piperaquine.[9,11] In the current study, gametocyte carriage persisted in all individuals treated with dihydroartemisinin–piperaquine-only until the end of follow-up (day 28) and three (16%) of 19 individuals were still infectious to mosquitoes 14 days after initiation of treatment. The addition of tafenoquine accelerated clearance of both male and female gametocytes in a dose-dependent manner; this is in line with reported in-vitro *P falciparum* gametocytocidal activity of tafenoquine.[5] Comparing the current findings with previous trials on the transmission-blocking effects of primaquine, wherein primaquine blocked transmission by day 2 post-treatment,[9,11] gametocyte clearance appears to be slower after tafenoquine, corroborating findings from a clinical trial in patients with *P vivax* in which tafenoquine had a longer time to gametocyte and parasite clearance

compared with chloroquine plus primaquine.[17] Contrary to observations with primaquine,[9,18] we also observed no evidence for an early sterilising effect of tafenoquine. Only by day 7 did we observe lower per-gametocyte infectivity in the tafenoquine treatment groups that we interpret as an indication that some gametocytes lose viability before their clearance; studies with more observations before day 7 are required to examine this further.[15] Our observation that the transmission-blocking activity of tafenoquine occurs later than day 2 is in line with findings from an avian *Plasmodium gallinaceum* model in which transmission to mosquitoes persisted for 4 days after initiation of tafenoquine monotherapy treatment and declined thereafter.[19] The reasons for this slow effect are unclear and could be related to both tafenoquine metabolism and activity. There is no consensus on the importance of CYP2D6-dependent tafenoquine activation: although rodent models indicate a relevant role for CYP2D6,[20,21] CYP2D6 intermediate metabolisers did not show lower tafenoquine activity against *P vivax* relapses[22] and CYP2D6-mediated activation thus appears less important for tafenoquine than for primaquine. Tafenoquine's activity against schizonts appears to be CYP2D6 independent,[23] but this does not rule out the possibility of CYP2D6 dependence for the drug's gametocytocidal activity. A generally lower activity of tafenoquine that only takes effect after several days of drug exposure might explain the late transmission-blocking properties of tafenoquine. More frequent assessments of infectivity in the first week after treatment would allow refinement of time of action.

Despite not having a direct comparison to dihydroartemisinin–piperaquine plus single low-dose primaquine, the current study suggests inferior transmission-blocking properties of the tested doses of tafenoquine compared with single low-dose primaquine tested in similar populations in multiple recent studies. Nonetheless, tafenoquine's long half-life could be a major advantage. Depending on the duration of its gametocytocidal activity, tafenoquine might play a role in preventing transmission of infections that are acquired after initiation of treatment. A long-acting gametocytocide could also be of relevance to prevent the transmission of drug-resistant parasites. Treatment failure has been associated with increased appearance of gametocytes after initiation of treatment,[24,25] and artemisinin-resistant gametocytes might have enhanced transmission potential under drug pressure.[26] A short-acting gametocytocidal drug such as primaquine delivered as a single dose is unlikely to affect gametocytes that appear in circulation after initiation of treatment, whereas tafenoquine might plausibly clear these gametocytes and thereby

prevent transmission of gametocytes related to delayed parasite clearance or recrudescence.

Whether low-dose gametocytocides are included in standard treatment or mass administrations, the choice of partner schizonticide requires careful consideration. The current study shows that dihydroartemisinin–piperaquine plus single low-dose tafenoquine has substantial gametocytocidal activity at doses of 1·66 mg/kg, but it is noteworthy that preliminary observations from an unpublished *P vivax* treatment trial indicate that that dihydroartemisinin–piperaquine might inhibit tafenoquine activity against *P vivax* hypnozoites.[27] Tafenoquine doses for *P vivax* are only recommended for use with chloroquine.[28] Given that chloroquine resistance is widespread for *P falciparum*, it seems logical that single low-dose tafenoquine will need to be combined with ACT. If the safety of these tafenoquine doses in G6PD mixed populations can be demonstrated, our data indicate this combination will be effective at clearing asexual parasites and transmission blockade.

To be used widely, tafenoquine's safety is a key consideration. We observed no increased number of adverse events in tafenoquine treatment groups compared with dihydroartemisinin–piperaquine-only. Non-symptomatic transient increases in alanine aminotransferase were observed in two individuals who received the lowest tafenoquine dose; this might be associated with general infection-related liver-injury that is treatment independent.[29] Due to the potentially greater risk of haemolysis in G6PD-deficient individuals,[30] our efficacy study population consisted only of adults with normal G6PD production. The distribution of G6PD deficiency worldwide is similar to that of malaria.[31] It is therefore essential that the minimally efficacious dose and safety thereof in individuals with normal G6PD production is established. No significant decreases in haemoglobin or increase in adverse events were observed in the tafenoquine groups compared with the dihydroartemisinin–piperaquine-only group. Moreover, the tafenoquine doses tested in our study population appear no less safe than single low-dose primaquine tested at the same location and with a similar population (ie, identical inclusion and exclusion criteria).[9] Although testing for G6PD deficiency is presently required before administration of tafenoquine for its current indications, future studies should explore the safety profile of single low doses of tafenoquine in larger populations that include participants who are G6PD deficient to inform the highest tolerable dose without previous G6PD testing. These studies should include a single low-dose comparator group. Future studies should also be

conducted in younger populations. Two previous studies with tafenoquine have been done in children. A study in Gabon with various tafenoquine doses for prophylaxis in semi-immune children and young adults between 12 and 20 years of age reported similar adverse events between treatment groups and compared with the placebo group but an average reduction in haemoglobin concentrations compared with baseline of 0·4 mg/dL with the highest tafenoquine dose of 200 mg.[32] Another phase 2 study assessed the safety and efficacy of chloroquine plus single weight-dependent doses of tafenoquine (100 mg, 150 mg, 200 mg, or 300 mg) in children aged between 2 and 15 years with *P vivax* malaria and G6PD enzyme activity of at least 70% in Columbia and Vietnam.[33] The most common adverse events attributed to drug treatment were vomiting and asymptomatic methaemoglobin increases. Haematological safety of tafenoquine in this study was similar to studies with (young) adults.

In the current study, we established the short-term effect of tafenoquine on transmission from highly infectious individuals. This population allows a detailed assessment of tafenoquine's transmission-blocking properties with high discriminative power. Our study shows that a dose of 1·66 mg/kg tafenoquine blocks transmission by day 7 but this effect might have been apparent at earlier moments when we did not do feeding experiments (days 3–7). In addition, our study provides no evidence for the duration of tafenoquine's transmission blocking activity. Future trials are required to examine the timing of tafenoquine activity with respect to both the initiation and duration of its *P falciparum* gametocytocidal activity. A design with a follow-up over multiple malaria episodes or in which tafenoquine is administered without an efficacious schizonticidal drug would allow assessment of the duration of tafenoquine's activity. With increasing evidence for the transmission-blocking effects of primaquine and tafenoquine in individual gametocyte carriers, there is an urgent need for community trials to establish the added value of these gametocytocides in first-line antimalarial treatment and in mass treatment campaigns to reduce the transmission of (drug-resistant) malaria.

Contributors

WS, AM, MJS, MBBM, TB, CD, and AD conceived the study and developed the study protocol. WS, AM, MJS, KS, YS, SMN, AS, SK, OMD, MD, SOM, SS, OA, TB, CD, and AD implemented the trial. KL did molecular analyses. WS and AM verified the raw data. WS and JB analysed the data. WS, AM, MJS, RtH, JB, TB, CD, and AD wrote the first draft of the manuscript. All authors read and

approved the final manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing

Anonymised data reported in the manuscript will be made available to investigators who provide a methodologically sound proposal to the corresponding author. The protocol is available upon request.

Declaration of interests

We declare no competing interests.

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References

1. WHO. World Malaria Report 2020. World Health Organization, Geneva, Switzerland, 2020.
2. WHO. Global Malaria Programme: WHO policy brief on single-dose primaquine as a gametocytocide in Plasmodium falciparum malaria. World Health Organization, Geneva, Switzerland, 2015.
3. Baird JK, Hoffman SL. Primaquine therapy for malaria. Clin Infect Dis 2004; 39: 1336-45.
4. Frampton JE. Tafenoquine: first global approval. Drugs 2018; 78: 1517-23.
5. Kemirembe K, Cabrera M, Cui L. Interactions between tafenoquine and artemisinin-combination therapy partner drug in asexual and sexual stage Plasmodium falciparum. Int J Parasitol Drugs Drug Resist 2017; 7: 131-37.
6. Coleman RE, Clavin AM, Milhous WK. Gametocytocidal and sporontocidal activity of antimalarials against Plasmodium berghei ANKA in ICR mice and Anopheles stephensi mosquitoes. Am J Trop Med Hyg 1992; 46: 169-82.
7. GlaxoSmithKline. Krintafel (tafenoquine) tablets, for oral use: US prescribing information. https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210795s000lbl.pdf (accessed Dec 7, 2021).
8. Rueangweerayut R, Bancone G, Harrell EJ, et al. Hemolytic potential of tafenoquine in female volunteers heterozygous for glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PD Mahidol variant) versus G6PD-normal volunteers. Am J Trop Med Hyg 2017; 97: 702-11.
9. Dicko A, Roh ME, Diawara H, et al. Efficacy and safety of primaquine and methylene blue for prevention of Plasmodium falciparum transmission in Mali: a phase 2, single-blind, randomised controlled trial. Lancet Infect Dis 2018; 18: 627-39.
10. Meerstein-Kessel L, Andolina C, Carrio E, et al. A multiplex assay for the sensitive detection and quantification of male and female Plasmodium falciparum gametocytes. Malar J 2018; 17: 441.
11. Dicko A, Brown JM, Diawara H, et al. Primaquine to reduce transmission of Plasmodium falciparum malaria in Mali: a single-blind, dose-ranging, adaptive randomised phase 2 trial. Lancet Infect Dis 2016; 16: 674-84.
12. Bousema T, Okell L, Shekalaghe S, et al. Revisiting the circulation time of Plasmodium falciparum gametocytes: molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. Malar J 2010; 9: 136.
13. Méndez F, Muñoz A, Plowe CV. Use of area under the curve to characterize transmission potential after antimalarial treatment. Am J Trop Med Hyg 2006; 75: 640-44.
14. WWARN Gametocyte Study Group. Gametocyte carriage in uncomplicated Plasmodium falciparum malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data. BMC Med 2016; 14: 79.
15. Bradley J, Soumaré HM, Mahamar A, et al. Transmission-blocking effects of primaquine and methylene blue suggest Plasmodium falciparum gametocyte sterilization rather than effects on sex ratio. Clin Infect Dis 2019; 69: 1436-39.
16. White NJ, Ashley EA, Recht J, et al. Assessment of therapeutic responses to gametocytocidal drugs in Plasmodium falciparum malaria. Malar J 2014; 13: 483.

17. Fukuda MM, Krudsood S, Mohamed K, et al. A randomized, double-blind, active-control trial to evaluate the efficacy and safety of a three day course of tafenoquine monotherapy for the treatment of Plasmodium vivax malaria. PLoS One 2017; 12: e0187376.
18. Stone W, Mahamar A, Sanogo K, et al. Pyronaridine–artesunate or dihydroartemisinin–piperaquine combined with single low-dose primaquine to prevent Plasmodium falciparum malaria transmission in Ouélessébougou, Mali: a four-arm, single-blind, phase 2/3, randomised trial. Lancet Microbe 2022; 3: e41-e51.
19. Tasai S, Saiwichai T, Kaewthamasorn M, et al. Artesunate-tafenoquine combination therapy promotes clearance and abrogates transmission of the avian malaria parasite Plasmodium gallinaceum. Vet Parasitol 2017; 233: 97-106.
20. Marcsisin SR, Sousa JC, Reichard GA, et al. Tafenoquine and NPC-1161B require CYP 2D metabolism for anti-malarial activity: implications for the 8-aminoquinoline class of anti-malarial compounds. Malar J 2014; 13: 2.
21. Vuong C, Xie LH, Potter BM, et al. Differential cytochrome P450 2D metabolism alters tafenoquine pharmacokinetics. Antimicrob Agents Chemother 2015; 59: 3864-69.
22. St Jean PL, Xue Z, Carter N, et al. Tafenoquine treatment of Plasmodium vivax malaria: suggestive evidence that CYP2D6 reduced metabolism is not associated with relapse in the phase 2b DETECTIVE trial. Malar J 2016; 15: 97.
23. Dow G, Smith B. The blood schizonticidal activity of tafenoquine makes an essential contribution to its prophylactic efficacy in nonimmune subjects at the intended dose (200 mg). Malar J 2017; 16: 209.
24. Barnes KI, Little F, Mabuza A, et al. Increased gametocytemia after treatment: an early parasitological indicator of emerging sulfadoxine-pyrimethamine resistance in falciparum malaria. J Infect Dis 2008; 197: 1605-13.
25. Spring MD, Lin JT, Manning JE, et al. Dihydroartemisinin–piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: an observational cohort study. Lancet Infect Dis 2015; 15: 683-91.
26. Witmer K, Dahalan FA, Delves MJ, et al. Transmission of artemisinin-resistant malaria parasites to mosquitoes under antimalarial drug pressure. Antimicrob Agents Chemother 2020; 65: e00898-e00920.
27. Baird JK, Sutanto I, Soebandrio A, et al. Evaluation of the efficacy and safety of tafenoquine co-administered with dihydroartemisinin–piperaquine for the radical cure (anti-relapse) of Plasmodium vivax malaria in Indonesia–INSPECTOR Study. American Society of Tropical Medicine & Hygiene 69th Annual Meeting; Nov 15–19, 2020 (abstr 1501).
28. Centers for Disease Control and Prevention: Global Health, Division of Parasitic Diseases and Malaria. Change in Krintafel (tafenoquine) label. https://www.cdc.gov/malaria/new_info/2020/tafenoquine_2020.html (accessed Dec 7, 2021).
29. Reuling IJ, de Jong GM, Yap XZ, et al. Liver injury in uncomplicated malaria is an overlooked phenomenon: an observational study. EBioMedicine 2018; 36: 131-39.
30. Shanks GD, Oloo AJ, Aleman GM, et al. A new primaquine analogue, tafenoquine (WR 238605), for prophylaxis against Plasmodium falciparum malaria. Clin Infect Dis 2001; 33: 1968-74.
31. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet 2008; 371: 64-74.
32. Lell B, Faucher JF, Missinou MA, et al. Malaria chemoprophylaxis with tafenoquine: a randomised study. Lancet 2000; 355: 2041-45.

33. Vélez ID, Hien TT, Green JA, et al. Taafenoquine exposure assessment, safety, and relapse prevention efficacy in children with Plasmodium vivax malaria: open-label, single-arm, non-comparative, multicentre, pharmacokinetic bridging, phase 2 trial. Lancet Child Adolesc Health 2022; 6: 86-95.

Supplementary Material

Please scan the QR code below to access the supplementary material for Chapter 2:

CHAPTER 3A

Artemether–lumefantrine with or without single-dose primaquine and sulfadoxine–pyrimethamine plus amodiaquine with or without single-dose tafenoquine to reduce *Plasmodium falciparum* transmission: a phase 2, single-blind, randomised clinical trial in Ouélessébougou, Mali

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Summary

Background

Artemether–lumefantrine is widely used for uncomplicated *Plasmodium falciparum* malaria; sulfadoxine–pyrimethamine plus amodiaquine is used for seasonal malaria chemoprevention. We aimed to determine the efficacy of artemether–lumefantrine with and without primaquine and sulfadoxine–pyrimethamine plus amodiaquine with and without tafenoquine for reducing gametocyte carriage and transmission to mosquitoes.

Methods

In this phase 2, single-blind, randomised clinical trial conducted in Ouelessebougou, Mali, asymptomatic individuals aged 10–50 years with *P falciparum* gametocytaemia were recruited from the community and randomly assigned (1:1:1:1) to receive either artemether–lumefantrine, artemether–lumefantrine with a single dose of 0·25 mg/kg primaquine, sulfadoxine–pyrimethamine plus amodiaquine, or sulfadoxine–pyrimethamine plus amodiaquine with a single dose of 1·66 mg/kg tafenoquine. All trial staff other than the pharmacist were masked to group allocation. Participants were not masked to group allocation. Randomisation was done with a computer-generated randomisation list and concealed with sealed, opaque envelopes. The primary outcome was the median within-person percent change in mosquito infection rate in infectious individuals from baseline to day 2 (artemether–lumefantrine groups) or day 7 (sulfadoxine–pyrimethamine plus amodiaquine groups) after treatment, assessed by direct membrane feeding assay. All participants who received any trial drug were included in the safety analysis. This study is registered with ClinicalTrials.gov, NCT05081089.

Findings

Between Oct 13 and Dec 16, 2021, 1290 individuals were screened and 80 were enrolled and randomly assigned to one of the four treatment groups (20 per group). The median age of participants was 13 (IQR 11–20); 37 (46%) of 80 participants were female and 43 (54%) were male. In individuals who were infectious before treatment, the median percentage reduction in mosquito infection rate 2 days after treatment was 100·0% (IQR 100·0–100·0; n=19; p=0·0011) with artemether–lumefantrine and 100·0% (100·0–100·0; n=19; p=0·0001) with artemether–lumefantrine with primaquine. Only two individuals who were infectious at baseline infected mosquitoes on day 2 after artemether–lumefantrine and none at day 5. By contrast, the median

percentage reduction in mosquito infection rate 7 days after treatment was 63·6% (IQR 0·0–100·0; n=20; p=0·013) with sulfadoxine–pyrimethamine plus amodiaquine and 100% (100·0–100·0; n=19; p<0·0001) with sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine. No grade 3–4 or serious adverse events occurred.

Interpretation

These data support the effectiveness of artemether–lumefantrine alone for preventing nearly all mosquito infections. By contrast, there was considerable post-treatment transmission after sulfadoxine–pyrimethamine plus amodiaquine; therefore, the addition of a transmission-blocking drug might be beneficial in maximising its community impact.

Research in context

Evidence before this study

We searched PubMed on June 13, 2023, with no restrictions on publication date or language, for studies assessing the post-treatment transmission of artemether–lumefantrine in combination with primaquine with search terms: "[Artemether-lumefantrine] OR [Coartem]" AND "[Primaquine] OR [Jasoprim] OR [Malirid] OR [Neo-Quipenyl] OR [Pimaquin] OR [Primachina] OR [Primacin] OR [Primaquina] OR [Remaquin]" AND "[Plasmodium falciparum]" AND "[Gametocytocidal] OR [Gametocytes]" AND "[Transmission]". An additional search was done for studies assessing the post-treatment transmission of sulfadoxine–pyrimethamine plus amodiaquine in combination with tafenoquine with the following search terms: "[Sulfadoxine-pyrimethamine plus amodiaquine] OR [Sulfadoxine-pyrimethamine with amodiaquine] OR "[Fansidar] AND ([Camoquin] OR [Flavoquine])" AND "[Tafenoquine] OR [Krintafel] OR [Arakoda] OR [WR238605]" AND "[Gametocytocidal] OR [Gametocytes]" AND "[Transmission]". Of the 19 results that fulfilled the initial search criteria, two studies conducted mosquito feeding assays following artemether–lumefantrine with primaquine treatment, yielding divergent outcomes. The remaining results encompassed the following: five studies that assessed safety or gametocyte carriage, or both, but did not include mosquito feeding assays, four studies that investigated different primaquine doses from the current study (0·1 mg/kg, 0·4 mg/kg, or 0·75 mg/kg, or a combination of these), and the remaining eight studies were not clinical trials. No results meeting the second search criteria were identified.

Added value of this study

Augmenting standard antimalarial treatments or mass administrations with a gametocytocidal drug might expedite efforts to achieve elimination and mitigate the spread of artemisinin-resistant *Plasmodium falciparum* strains. For specific treatment recommendations to be made, it is crucial we know how transmission potential is affected by distinct antimalarial therapies. Evaluating post-treatment transmission potential based solely on gametocyte carriage is inadequate because transmissible gametocytes might persist at submicroscopic densities following treatment, and certain antimalarial drugs can render gametocytes non-viable before their elimination from the circulation. Such assessments, therefore, require mosquito-feeding assays. Our study provides robust evidence that artemether–lumefantrine has potent transmission-blocking activity, even in the absence of primaquine. We also provide the first direct evidence of substantial transmission beyond a week after treatment with sulfadoxine–pyrimethamine plus amodiaquine. Notably, we provide the first data demonstrating that transmission after sulfadoxine–pyrimethamine plus amodiaquine can be annulled by administration of a single low dose of tafenoquine. Using direct assays of transmission and highly sensitive sex-specific gametocyte quantification, this investigation contributes to our understanding of the transmission-blocking activity of artemether-lumefantrine and strongly supports the incorporation of a gametocytocidal drug in combination with sulfadoxine–pyrimethamine plus amodiaquine.

Implications of all the available evidence

The gametocytocidal effects of artemether-lumefantrine observed in this study are consistent with the findings of earlier studies, suggesting that the inclusion of single low-dose primaquine might not be necessary when administering artemether–lumefantrine as antimalarial treatment in these age groups in areas where WHO recommends the addition of single low-dose primaquine. Growing concerns for the sustainability of artemether–lumefantrine as a first-line treatment might increase the utility of supplemental gametocytocides in future, either as part of standard treatments or in mass administrations. Our uniquely direct evidence of continued transmission following sulfadoxine–pyrimethamine plus amodiaquine is in agreement with previous observations of prolonged post-treatment gametocytaemia. These data strongly suggest that the community-wide benefits of seasonal malaria chemoprevention could be enhanced by the addition of gametocytocidal drug such as primaquine or tafenoquine.

The community benefit of mass administration with 8-aminoquinolines for *P falciparum* transmission reduction is, to date, untested.

Introduction

Artemisinin-based combination therapies (ACTs) retain excellent efficacy for treatment of uncomplicated malaria in Africa, despite concerning evidence for reduced sensitivity of parasites in east Africa to artemisinins.[1,2] ACTs rapidly clear asexual parasites but have variable activity against mature gametocytes that are the parasite life stage that can be transmitted to mosquitoes. A pooled analysis of post-treatment microscopy data indicated that artemether–lumefantrine might be the most potent ACT in terms of gametocyte clearance whereas gametocyte persistence is markedly longer after the drug combination dihydroartemisinin–piperaquine.[3] Importantly, post-treatment gametocyte carriage is an imperfect approximation of malaria transmission potential. Transmissible gametocytes might persist at submicroscopic densities after treatment and some antimalarial drugs might sterilise gametocytes before these are removed from the circulation.[4] Although artemether–lumefantrine is the most widely used treatment regimen for uncomplicated *Plasmodium falciparum* infections globally,[5] data on malaria transmission after artemether–lumefantrine treatment are conflicting. Five studies that performed mosquito feeding assays after artemether–lumefantrine treatment reached different conclusions. Three studies observed non-negligible transmission between 7 days and 14 days after treatment with artemether–lumefantrine[6,7] or artemether–lumefantrine plus primaquine,[8] whereas two others found near complete abrogation of transmission 1 week after initiation of artemether–lumefantrine treatment without primaquine.[9,10] The only study that assessed transmission to mosquitoes before and after artemether–lumefantrine treatment saw only one (2%) of 49 individuals infect mosquitoes at day 7 after treatment initiation, with mosquito infection rates markedly reduced compared with pretreatment rates.[9] Quantifying post-treatment transmission potential after the most commonly used antimalarial is important: if substantial transmission occurs after treatment with artemether–lumefantrine, the addition of a specific gametocytocide might need to be considered. WHO recommends that to reduce onward transmission of *P falciparum*, ACTs might be combined with a single low dose of primaquine (0·25 mg/kg),[11] which has potent but short lived gametocytocidal activity. Although this recommendation is currently limited to areas of low transmission, it has received new attention in sub-Saharan Africa since the emergence of

artemisinin resistance in Africa.[12] As a *P falciparum* gametocytocide, one low dose of 0·25 mg/kg primaquine, when combined with ACTs, causes an immediate and effective reduction in the transmission of parasites[13,14] and is safe for use without previous glucose-6-phosphate dehydrogenase (G6PD) status testing.[15] Whether the addition of primaquine to artemether–lumefantrine is beneficial or whether artemether–lumefantrine already sufficiently prevents post-treatment transmission is currently unclear.

In addition, a study with tafenoquine, which is being developed as a long-lasting alternative to primaquine for preventing *Plasmodium vivax* relapse,[16] demonstrated that one low dose of tafenoquine (1·66 mg/kg) in combination with dihydroartemisinin–piperaquine completely prevented infectivity within 7 days after initiation of treatment in Malian adults and children.[17] Although this trial showed that tafenoquine can prevent transmission when co-administered with dihydroartemisinin–piperaquine, the effect appeared to be slower than primaquine.[18] The combination of tafenoquine with non-ACTs to clear *P falciparum* gametocytes and prevent transmission has not been tested. Sulfadoxine–pyrimethamine plus amodiaquine, a non-artemisinin-based combined anti-malarial treatment, is the only antimalarial recommended for systematic mass administration in the form of seasonal malaria chemoprevention.[19] As per WHO guidance, a non-ACT regimen is used for seasonal malaria chemoprevention due to the precaution that first-line or second-line malaria treatments should not be used for chemoprevention within the same country.[20] Previous studies showed considerable post-treatment transmission potential following sulfadoxine–pyrimethamine plus amodiaquine administration, with infectivity and mosquito infection rate unaffected for the first 7 days after treatment and reductions in gametocytaemia only observed after 28 days.[7,14] Increased gametocyte densities in the first 2 weeks following sulfadoxine–pyrimethamine plus amodiaquine will limit any effect of seasonal malaria chemoprevention on transmission.[7] The combination of sulfadoxine–pyrimethamine plus amodiaquine with a single low dose of tafenoquine has never been tested.

In the current study, we aimed to assess the reduction of infectivity of *P falciparum* gametocytes following administration of the two widely used malaria treatments artemether–lumefantrine and sulfadoxine–pyrimethamine plus amodiaquine in combination with a single low dose of the gametocytocidal drugs primaquine and tafenoquine, respectively, in Malian children and adults.

Methods

Study design and participants

This four-group, single-blind, phase 2, randomised trial was conducted at the Ouelessebougou Clinical Research Unit of the Malaria Research and Training Centre (MRTC) of the University of Bamako in Mali. Ouelessebougou is a commune that includes the town of Ouelessebougou and 44 surrounding villages, with an estimated 50 000 inhabitants. Situated roughly 80 km to the south of Bamako, the capital of Mali, this area has a distinct seasonality in malaria transmission due to the rainy season from July to November. The prevalence of *P falciparum* malaria and gametocytes in children older than 5 years varies between 50% to 60% and 20% to 25%, respectively, during the transmission season. Before the commencement of screening, the study team met with community leaders, village health workers, and heads of households from each village to explain the study and obtain approval to conduct the study. Village health workers then used a door-to-door approach to inform households of the date when, and location where, consenting and screening would take place. Participants were included in the trial if they met the following criteria: positive for *P falciparum* gametocytes by microscopy (ie, ≥1 gametocytes recorded in a thick film against 500 white blood cells, equating to 16 gametocytes per µL with a standard conversion of 8000 white blood cells per µL blood); absence of other non-*P falciparum* species on blood film; haemoglobin density of at least 10 g/dL; G6PD normal (male >4 IU/g haemoglobin, female >6 IU/g haemoglobin); aged 10–50 years; bodyweight of 80 kg or less; no clinical signs of malaria, defined by fever (≥37·5°C); no signs of acute, severe, or chronic disease; and, consistent with the long half-life of tafenoquine, use of effective contraception for five half-lives (3 months) after the end of tafenoquine treatment. Exclusion criteria included pregnancy (tested at enrolment by urine or serum test, or both) or lactation, allergies to any of the study drugs, use of other medication (except for paracetamol or aspirin, or both), use of antimalarial drugs over the past week, signs of acute or chronic illness, history of psychiatric disorders, and blood transfusion in the past 90 days. A detailed list of inclusion and exclusion criteria is provided in the appendix. Before screening and study enrolment, participants provided written informed consent (≥18 years) or assent with written parental consent (10–17 years).

Ethical approval was granted by the ethics committee of the Faculty of Medicine, Pharmacy, and Dentistry of the University of Science, Techniques,

and Technologies of Bamako (Bamako, Mali; number 2021/189/CE/USTTB), and the research ethics committee of the London School of Hygiene and Tropical Medicine (London, UK; reference number 26257). The trial protocol is available in the appendix. The trial is registered with ClinicalTrials.gov, NCT05081089.

Randomisation and masking

Allocation to four treatment groups (artemether–lumefantrine, artemether–lumefantrine with primaquine [0·25 mg/kg], sulfadoxine–pyrimethamine plus amodiaquine, and sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine [1·66 mg/kg]) was randomised in a 1:1:1:1 ratio. Enrolment continued until 80 participants were enrolled (20 individuals assigned to each treatment group). An independent MRTC statistician randomly generated the treatment assignment using Stata, version 16, which was linked to participant identification numbers. The statistician prepared sealed, opaque envelopes with the participant identification number on the outside and treatment assignment inside, which were sent to the MRTC study pharmacist. The study pharmacist provided treatment and was consequently not masked to treatment assignment; staff involved in assessing safety and laboratory outcomes were masked to group allocation. Participants were not masked to group allocation.

Procedures

Artemether–lumefantrine treatment (Novartis, Basel, Switzerland) was administered over 3 days as per manufacturer instructions (supplementary information 1). A single dose of 0·25 mg/kg primaquine (ACE Pharmaceuticals, Zeewolde, Netherlands) was administered on day 0 in parallel with the first dose of artemether–lumefantrine, as described previously.[14] Participants in the sulfadoxine–pyrimethamine plus amodiaquine groups were treated with standard doses of sulfadoxine–pyrimethamine plus amodiaquine (Guilin Pharmaceutical, Shanghai, China) as per manufacturer instructions (supplementary information 1). A single dose of tafenoquine (60° pharmaceuticals, Washington DC, USA) was administered on day 0 in parallel with the first doses of sulfadoxine–pyrimethamine and amodiaquine. Tafenoquine dosing was weight based to standardise efficacy and risk variance (supplementary information 1). The dose of 1·66 mg/kg was chosen on the basis of the good safety profile and efficacy in Malian adults and children aged at least 12 years.[17] G6PD testing was conducted using both semi-quantitative (OSMMR-D G-6-PD test; R&D Diagnostics, Aghia Paraskevi, Greece) and quantitative testing (STANDARD G6PD Test; SD BIOSENSOR, Suwon,

South Korea); inclusion in the study required normal enzyme function to be determined by both methods.

Participants received a full clinical and parasitological examination on days 2, 5, 7, 14, 21, and 28 after receiving the first dose of the study drugs (supplementary figure 1). Giemsa-stained thick film microscopy was performed as described previously, with asexual stages counted against 200 white blood cells and gametocytes counted against 500 white blood cells.[14] For molecular gametocyte quantification, total nucleic acids were extracted using a MagNAPure LC automated extractor (Total Nucleic Acid Isolation Kit-High Performance; Roche Applied Science, Indianapolis, IN, USA). Male and female gametocytes were quantified in a multiplex rRT-qPCR assay (supplementary table 1).[21] Samples were classified as negative for a particular gametocyte sex if the qRT-PCR quantified density of gametocytes of that sex was less than 0·01 gametocytes per µL (ie, one gametocyte per 100 µL of blood sample). Haemoglobin density was measured using a haemoglobin analyser (HemoCue; AB Leo Diagnostics, Helsingborg, Sweden) or automatic haematology analyser (HumaCount 5D; Wiesbaden, Germany). Additional venous blood samples were taken for biochemical and infectivity assessments on days 0, 2, 5, 7, and 14 in all treatment groups. Aspartate transaminase, alanine transaminase, and blood creatine levels were determined using automatic biochemistry analyser Human 100 (Wiesbaden, Germany). For each assessment of infectivity, about 75 female *Anopheles gambiae* mosquitoes distributed into three cups, locally reared at the insectary, were allowed to feed for 15–20 min on venous blood samples (Lithium Heparin VACUETTE tube; Greiner Bio-One, Kremsmünster, Austria) through a prewarmed glass membrane feeder system (Coelen Glastechniek; Weldaad, Netherlands). Surviving mosquitoes were dissected on the seventh day after feeding; midguts were stained in 1% mercurochrome and examined for the presence and density of oocysts by expert microscopists.

Outcomes

The primary outcome was the median within-person percent change in mosquito infection rate in infectious individuals from baseline to day 2 (artemether–lumefantrine groups) or from baseline to day 7 (sulfadoxine–pyrimethamine plus amodiaquine groups) after treatment. Secondary outcomes were mosquito infection metrics (oocyst density, mosquito infection rate, and infectivity to mosquitoes), gametocyte metrics (prevalence, density, circulation time, area under the curve [AUC] of density over time, and sex ratio

[ie, proportion of gametocytes that were male]), and asexual and total parasite prevalence and density compared within treatment groups to baseline, at all feeding timepoints (days 2, 5, 7, 14, 21, and 28), and between treatment-matched groups. Primary and secondary analyses of mosquito infection rate and oocyst density metrics were performed on individuals infectious at baseline but are presented for all individuals in supplementary table 2 and 3. Gametocyte infectivity was assessed as an exploratory outcome.

Safety assessments included haemoglobin and methaemoglobin density and median within-person percent change in density, and incidence of clinical and laboratory adverse events. Adverse events were graded by the study clinician for severity (mild, moderate, or severe) and relatedness to study medication (unrelated or unlikely, possibly, probably, or definitely related). A drop in haemoglobin concentration of 40% or more from baseline was categorised as a haematological severe adverse event. An external data safety and monitoring committee was assembled before the trial, and safety data were discussed after enrolment of 40 participants, and after the final follow-up visit of the last participant.

Statistical analysis

Sample size estimation was based on infectivity for participants in previous trials in the same study setting using a mixed-effects logistic regression model that accounted for correlation between mosquito observations from the same participant.[13,14,17,18] With an expected reduction in infectivity of 90% as previously detected for efficacious doses of primaquine and tafenoquine,[14,17,18] we calculated 92% empirical power to detect more than 85% reduction in infectivity with a one-tailed test with a level of significance α of 0·05 when including 20 participants and dissecting 50 mosquitoes at each timepoint. When using an α of 0·025, empirical power was calculated at 90%. The sample size was not designed to compare the transmission-blocking effects between treatment groups; comparison of outcomes between groups was secondary and was only undertaken for treatment matching groups.

Clinical and entomological data were double entered into a Microsoft Access (version 365) database and analysed using Stata (version 16.0) and SAS (version 9.4). Statistical analyses of mosquito infection rate and oocyst density were analysed at timepoints after baseline only for those individuals who were infectious at baseline. The prevalence of gametocytes and infectious individuals were compared within and between treatment groups using one-

sided Fisher's exact tests. Haemoglobin concentrations were compared using paired *t* tests (*t* score) for within-group analyses and linear regression adjusted for baseline levels of each measure for between-group analyses (*t* score, coefficient with 95% CI). Percentage change from baseline was analysed using two-way *t* tests. The proportion of gametocytes that were male was analysed for all values with total gametocyte densities of 0·2 gametocytes per µL or more.[14] Gametocyte circulation time was calculated to determine the mean number of days that a mature gametocyte circulates in the blood before clearance using a deterministic compartmental model that assumes a constant rate of clearance and has a random effect to account for repeated measures on individuals, as described previously.[22] Difference in circulation time between groups and between gametocyte sexes was analysed using *t* tests (*t* score). AUC of gametocyte density per participant over time was calculated using the linear trapezoid method and was analysed by fitting linear regression models to the $\log_{10}$-adjusted AUC values, with adjustment for baseline gametocyte density (*t* score, coefficient with 95% CI). All other analyses of quantitative data were done using Wilcoxon sign rank tests (Z score) and Wilcoxon rank-sum tests (Z score). All comparisons were defined before study completion and analyses were not adjusted for multiple comparisons. Gametocyte infectivity was assessed using logistic regression models adjusted for gametocyte density, wherein the shape of the relationship between gametocyte density and mosquito infection rate was estimated using fractional polynomials. The outcome measure of this analysis was odds ratio (95% CI). For all analyses, the threshold for statistical significance was set at $p<0·05$.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Oct 13 and Dec 16, 2021, 1290 individuals were assessed for eligibility and 80 were enrolled and randomly assigned to one of the four treatment groups (n=20 per group), of which 77 (96%) completed all follow-up visits (figure 1). Participant baseline characteristics were similar between the study groups (table 1). Overall, median age was 13 years (IQR 11–20); 37 (46%) of 80 participants were female and 43 (54%) were male.

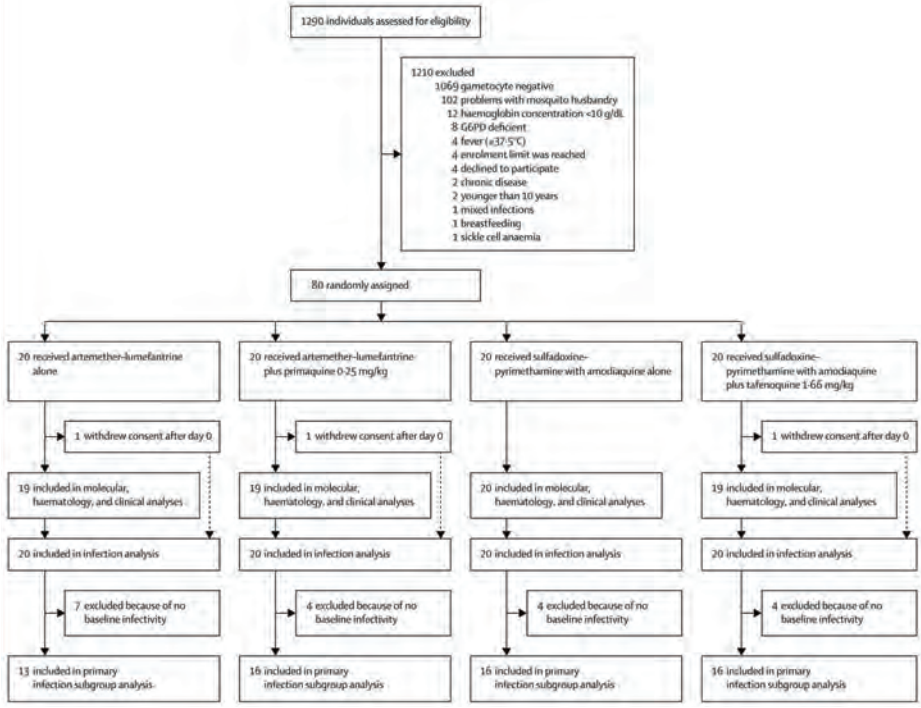

Figure 1. Trial profile.

Before treatment, 61 (76%) of 80 participants were infectious to mosquitoes, with a median of 9·7% (IQR 4·2–20·7) of mosquitoes becoming infected (table 2; supplementary table 2). At day 2, two (11%) of 19 participants in the artemether–lumefantrine group and zero (0%) of 19 participants in the artemether–lumefantrine with primaquine group infected mosquitoes (figure 2). In individuals who were infectious before treatment, the median percentage reduction in mosquito infection rate 2 days after treatment was 100·0% (IQR 100·0–100·0) for individuals treated with artemether–lumefantrine (n=19; p=0·0011) and 100·0% (IQR 100·0–100·0) with artemether–lumefantrine with primaquine (n=19; p=0·0001). Two participants (one in the sulfadoxine–pyrimethamine plus amodiaquine group and one in the sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine group) were infectious on days 2 and 5, but not infectious on day 0. Furthermore, two participants (one participant in the artemether–lumefantrine group and one in the sulfadoxine–pyrimethamine plus amodiaquine group) were infectious on day 5, but not infectious on days 0 or 2. In addition, one participant in the artemether–lumefantrine with primaquine group was infectious at baseline and

	Artemether–lumefantrine (n=20)	Artemether–lumefantrine with primaquine (n=20)	Sulfadoxine–pyrimethamine plus amodiaquine (n=20)	Sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine (n=20)
Age, years	14 (12–19)	12 (10–19)	14 (12–22)	13 (10–20)
Female	12 (60%)	7 (35%)	11 (55%)	7 (35%)
Male	8 (40%)	13 (65%)	9 (45%)	13 (65%)
Weight, kg	37·5 (30·0–49·6)	35·5 (27·3–48·3)	46·5 (31·0–58·0)	35·5 (25·0–55·5)
Haemoglobin, g/dL	11·5 (11·0–12·3)	12·4 (12·0–12·9)	12·4 (11·2–13·3)	12·8 (12·1–14·0)
Gametocyte prevalence	20 (100%)	20 (100%)	20 (100%)	20 (100%)
Gametocyte density, parasites per µL	41·3 (9·9–58·4)	30·4 (27·6–92·4)	36·4 (16·1–145·4)	24·5 (7·7–126·0)
Asexual parasite prevalence	12 (60%)	11 (55%)	12 (60%)	11 (55%)
Asexual parasite density, parasites per µL	400 (120–2340)	400 (120–1120)	440 (180–2060)	400 (80–2160)

Table 1. Baseline characteristics. Data are median (IQR) or n (%).

at day 5, but not at day 2. Post-treatment infectivity in these cases might stem from variations in drug susceptibility or exposure during distinct gametocyte development stages. At day 7, 11 (55%) of 20 participants in the sulfadoxine–pyrimethamine plus amodiaquine group and zero (0%) of the 19 participants in the sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine group infected any number of mosquitoes. In individuals who were infectious before treatment, the median percentage reduction in mosquito infection rate 7 days after treatment was 63·6% (IQR 0·0–100·0) for individuals treated with sulfadoxine–pyrimethamine plus amodiaquine (n=11; p=0·013) and 100·0% (IQR 100·0–100·0) for individuals treated with sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine (n=19; p<0·0001).

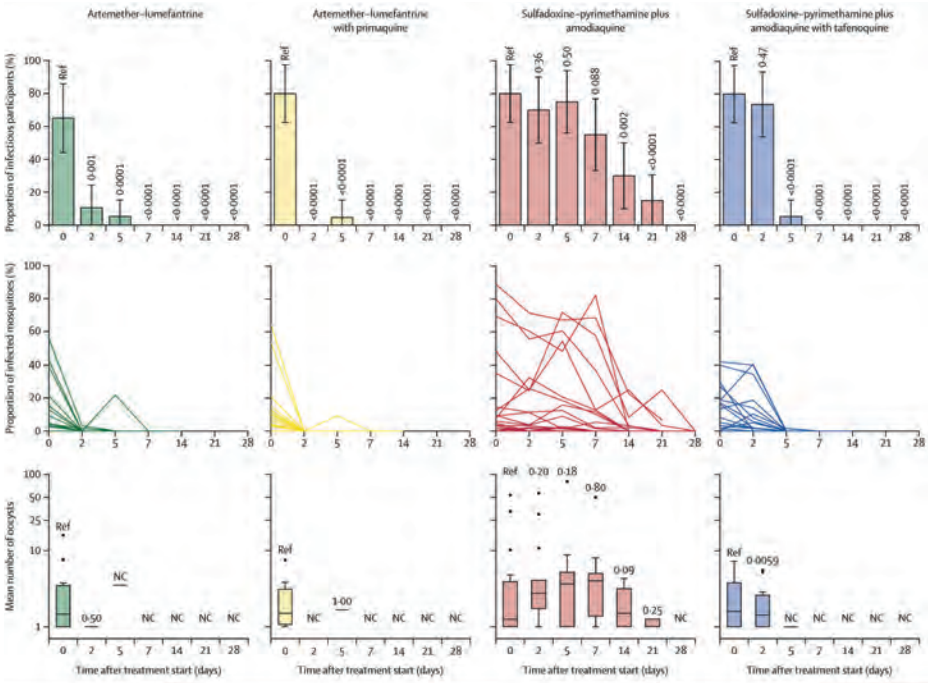

Figure 2. Participant infectivity and proportion of mosquitoes infected indirect membrane feeding assays. For participant infectivity, p values are calculated from generalised linear models testing differences within treatment groups, with baseline as reference; error bars are 95% CI. The denominator for the proportion of infectious participants is the total number of participants still enrolled at a given timepoint, rather than the number tested at that timepoint for infectivity. For mosquito infection rate, each line represents one participant. For oocyst density, Wilcoxon signed-rank tests for differences in average oocyst density are shown. Box plots show the median (central line), IQR (box limits), upper and lower quartiles plus 1·5 × IQR (whiskers), and outliers for mean oocyst densities in infected mosquitoes within each participant. NC=not calculable.

Table 2. Infectivity to mosquitoes before and after treatment.

	Infectious individuals*	Mosquito infection rate†	Reduction in mosquito infection rate‡	Within-group	Between-group
Pretreatment					
Artemether–lumefantrine	13/20 (65%)	9·4% (4·2 to 21·3)	..	..	..
Artemether–lumefantrine plus primaquine	16/20 (80%)	9·8% (5·3 to 13·8)	..	..	..
Sulfadoxine–pyrimethamine and amodiaquine	16/20 (80%)	9·3% (4·0 to 41·8)	..	..	..
Sulfadoxine–pyrimethamine and amodiaquine plus tafenoquine	16/20 (80%)	14·8% (3·2 to 23·0)	..	..	..
Day 2					
Artemether–lumefantrine	2/19 (11%)	0% (0 to 0)	100·0% (100·0 to 100·0)	0·0011	Ref
Artemether–lumefantrine plus primaquine	0/19 (0%)	0% (0 to 0)	100·0% (100·0 to 100·0)	0·0001	0·11
Sulfadoxine–pyrimethamine and amodiaquine	14/20 (70%)	11·4% (2·3 to 29·2)	26·0% (0·8 to 54·5)	0·11	Ref
Sulfadoxine–pyrimethamine and amodiaquine plus tafenoquine	14/19 (74%)	3·4% (1·5 to 16·4)	59·6% (9·3 to 84·5)	0·088	0·30
Day 5					
Artemether–lumefantrine	1/19 (5%)	0% (0 to 0)	100·0% (100·0 to 100·0)	0·0005	Ref
Artemether–lumefantrine plus primaquine	1/19 (5%)	0% (0 to 0)	100·0% (100·0 to 100·0)	0·0047	0·37
Sulfadoxine–pyrimethamine and amodiaquine	15/20 (75%)	14·2% (1·4 to 51·2)	24·5% (−49·3 to 71·1)	0·57	Ref
Sulfadoxine–pyrimethamine and amodiaquine plus tafenoquine	1/19 (5%)	0% (0 to 0)	100·0% (100·0 to 100·0)	0·0001	<0·0001

	Infectious individuals*	Mosquito infection rate†	Reduction in mosquito infection rate‡	Within-group	Between-group
Day 7					
Artemether–lumefantrine	0/19 (0%)	0% (0 to 0)	100·0% (100·0 to 100·0)	0·0005	Ref
Artemether–lumefantrine plus primaquine	0/19 (0%)	0% (0 to 0)	100·0% (100·0 to 100·0)	0·0001	1·0
Sulfadoxine–pyrimethamine and amodiaquine	11/20 (55%)	7·8% (0·0 to 24·9)	63·6% (0·0 to 100·0)	0·013	Ref
Sulfadoxine–pyrimethamine and amodiaquine plus tafenoquine	0/19 (0%)	0% (0 to 0)	100·0% (100·0 to 100·0)	0·0001	0·0001

Table 2. Infectivity to mosquitoes before and after treatment. Data are n/N (%) or median (IQR), unless stated otherwise. Full details of mosquito feeding assay outcomes are in the supplementary table 2 and 3.

* Individuals were classed as infectious if direct membrane feeding assays resulted in at least one mosquito with any number of oocysts. Mosquito infection measures (percentage infection and oocyst density) are shown for all participants who were infectious at baseline, and oocyst densities are from all infected mosquitoes.

† Median proportion of mosquitoes infected by each participant, where for each participant the mosquito infection rate was the number of mosquitoes infected as a proportion of all mosquitoes surviving to dissection.

‡ Median reduction (relative to baseline) in mosquito infection rate at the given timepoints. All values are for individuals who were infectious to mosquitoes before treatment (ie, infected any number of mosquitoes).

The median reduction in mosquito infection rate in the artemether–lumefantrine group was not significantly different from the artemether–lumefantrine with primaquine group at any timepoint. No mosquito infections were observed after day 5 in either artemether–lumefantrine treatment groups. In the sulfadoxine–pyrimethamine plus amodiaquine and sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine groups, respectively, 14 (70%) of 20 and 14 (74%) of 19 individuals were infectious to mosquitoes at day 2, whereas 15 (75%) of 20 and one (5%) of 19 were infectious at day 5. Median reduction in mosquito infection rate in individuals infectious at baseline was significantly different between groups by day 5, when there was a 24·5% (IQR −49·3 to 71·1) reduction in the sulfadoxine–pyrimethamine plus amodiaquine group, and near total abrogation of infection with the addition of tafenoquine (100·0%, IQR 100·0 to 100·0; figure 2; table 2). In the sulfadoxine–pyrimethamine plus amodiaquine group, 11 (69%) of 16 individuals infectious to mosquitoes at baseline were still infectious a week later, and 3 weeks after treatment three (19%) of 16 remained infectious. The median oocyst density on day 2 in the sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine group was significantly different from day 0 (p=0·0059). No other significant differences were found in median oocyst density within or between groups at any of the timepoints (figure 2; supplementary table 2). Mosquito infection measures for all participants (regardless of baseline infectivity) are shown in the supplementary table 3.

Gametocyte densities declined after initiation of treatment in all treatment groups, although the decrease was much less rapid in the sulfadoxine–pyrimethamine plus amodiaquine group than in any of the other groups (figure 3; supplementary table 5 and 7). All 20 (100%) participants treated with sulfadoxine–pyrimethamine plus amodiaquine alone remained gametocyte positive on the final day of observation (day 28), whereas 11 (58%) of 19 who received artemether–lumefantrine alone were still gametocyte positive at that same point. Total gametocyte circulation time was estimated at 5·3 days (95% CI 4·5–6·0) in the artemether–lumefantrine group and 2·9 days (2·4–3·3) in the artemether–lumefantrine with primaquine group (supplementary table 4); the same measure was estimated at 9·1 days (7·3–11·0) and 3·3 days (2·9–3·6) in the sulfadoxine–pyrimethamine plus amodiaquine and sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine groups, respectively. Gametocyte sex ratios were initially similar in all treatment groups, but the ratio increased towards male in the artemether–lumefantrine group from day 2 after treatment (median proportion of male gametocytes 0·46 [IQR 0·37–0·51]

on day 0 and 0·80 [0·60–0·92] on day 2; p=0·013]; supplementary table 5 and 7, supplementary figure 2). In the sulfadoxine–pyrimethamine plus amodiaquine group, sex ratios remained largely unchanged but with an increasing bias toward females over the 28-day trial period. Treatment with primaquine and tafenoquine resulted in significantly male-biased ratios from day 5 (p=0·0023 and p<0·0001, respectively). The infectivity of persisting gametocytes was significantly lower in the sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine group than in the sulfadoxine–pyrimethamine plus amodiaquine group (day 2 odds ratio [OR] 0·59 [95% CI 0·44–0·79], p<0·0001; day 5 OR 0·0077 [0·0011–0·056], p<0·0001), whereas in the artemether–lumefantrine groups too few gametocytes persisted to allow this comparison (supplementary table 8).

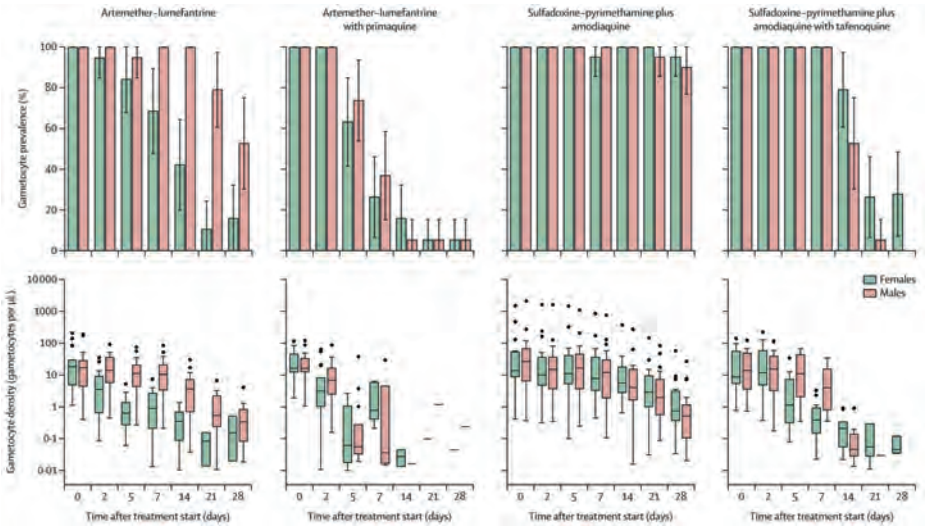

Figure 3. Male and female gametocyte density and prevalence. Gametocyte prevalence estimates are shown with 95% CIs. Gametocyte density is shown for gametocyte-positive individuals only (ie, male or female density >0·01 per µL). Box plots show the median (central line), IQR (box limits), upper and lower quartiles plus 1·5 × IQR (whiskers), and outliers.

There were no haemolytic severe adverse events (ie, drop of >40% from baseline). Transient reductions in haemoglobin density were greater in the sulfadoxine–pyrimethamine groups than in the artemether–lumefantrine groups, with significant reductions in haemoglobin density observed at days 1 and 2 (during the period of treatment administration) in both sulfadoxine–pyrimethamine plus amodiaquine groups (mean change −5·0%); these resolved after day 5 (supplementary table 9 and supplementary figure 3). The maximum drop in haemoglobin between baseline and any study timepoint

	All (n=80)	Artemether–lumefantrine (n=20)	Artemether–lumefantrine with primaquine (n=20)	Sulfadoxine–pyrimethamine plus amodiaquine (n=20)	Sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine (n=20)
All	50 (62·5%)	6 (30%)	13 (65%)	16 (80%)	15 (75%)
p value	0·16*	..	0·056†	..	1·00†
Mild related adverse event	29 (36%)	4 (20%)	9 (45%)	10 (50%)	6 (30%)
p value	0·37*	..	0·18†	..	0·33†
Moderate related adverse event	9 (11%)	0	0	3 (15%)	6 (30%)
p value	0·012*	..	**nc**	..	0·45†
Severe related adverse event	0	0	0	0	0
p value	nc	..	**nc**	..	nc

Table 3. Frequency of adverse events. Data are n (%). If there were multiple episodes per participant, the highest grade is presented. Classification as related to treatment was defined as probably, possibly, or definitely related to treatment, as described in the methods. NC=not calculable.

* p values are from Fisher's exact tests for differences in proportion of individuals with an adverse event between all groups.

† p values are from Fisher's exact tests for differences in proportion of individuals with an adverse event between artemether–lumefantrine with primaquine group and the artemether–lumefantrine reference group and sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine group and the sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine reference group.

was 15·8% in the artemether–lumefantrine group, 15·9% in the artemether–lumefantrine with primaquine group, 24·3% in the sulfadoxine–pyrimethamine plus amodiaquine group and 23·5% in the sulfadoxine–pyrimethamine plus amodiaquine with tafenoquine group. The concentration in blood methaemoglobin was significantly higher in the artemether–lumefantrine with primaquine group compared with the artemether–lumefantrine group at day 1 (1·8 [0·5–3·0] *vs* 1·5 [0·6–2·3]; p=0·010) and in the sulfadoxine–pyrimethamine with amodiaquine plus tafenoquine group compared with the sulfadoxine–pyrimethamine with amodiaquine group on day 2 (1·8 [1·4–2·5] *vs* 1·5 [0·5–2·3]; p=0·029) and day 5 (1·9 [0·9–3·0] *vs* 1·6 [1·0–2·4]; p=0·020; supplementary table 10 and supplementary figure 3).

Overall, 50 (63%) of the 80 participants experienced a total of 92 adverse events during follow-up, of which 61 were at least possibly related to the study drugs. 48 (79%) of the at least possibly related adverse events were categorised as mild and 13 (21%) as moderate. No grade 3 or serious adverse events occurred (table 3). The most common adverse events were headaches, abdominal pain, and nausea. No severe laboratory abnormalities occurred; all possibly drug-related laboratory abnormalities normalised on the subsequent visit (supplementary table 11).

Discussion

In this randomised trial, we determined the gametocytocidal and transmission-blocking activities of the most widely used first-line antimalarial, artemether–lumefantrine, with and without the WHO-recommended single low dose of 0·25 mg/kg of primaquine, and the preferred chemopreventative treatment, sulfadoxine–pyrimethamine plus amodiaquine, with and without one dose of 1·66 mg/kg tafenoquine. Artemether–lumefantrine alone blocked almost all transmission within 2 days of treatment initiation, whereas sulfadoxine–pyrimethamine plus amodiaquine had little effect on gametocyte density and prevalence, with significant reductions in infectivity to mosquitoes only observed after 14 days. Although the addition of tafenoquine did not prevent transmission at day 2 after treatment initiation, no infected mosquitoes were observed at the next mosquito feeding timepoint on day 5.

Calls for malaria eradication and concerns about the spread of drug resistance have increased interest in the effects of antimalarial drugs on gametocytes

and their infectiousness.[23] Since transmission cannot be reliably predicted from gametocyte densities and direct mosquito transmission outcomes are technically challenging, assessments of post-treatment infectiousness have been few and inconsistent. Previous studies demonstrated that the gametocytocidal effect of artemether–lumefantrine is superior to that of other ACTs, such as dihydroartemisinin–piperaquine and pyronaridine–artesunate,[24,25] but were inconclusive on its ability to prevent transmission to mosquitoes early after treatment.[6–10] In a selected population with high gametocyte densities and confirmed transmission potential before treatment, we demonstrate that artemether–lumefantrine exerts a strong gametocytocidal and transmission-blocking effect. Our finding of a median within-person reduction in mosquito infection rate of 100% 2 days after artemether–lumefantrine treatment corroborates findings from clinical trials in Kenya and The Gambia.[6,10] Gametocyte densities also declined after artemether–lumefantrine, with drop of 44% (38·44 [IQR 9·15–56·47] to 21·61 [7·51–44·01] gametocytes per µL) between baseline and day 2. However, participants were still carrying theoretically transmissible densities of gametocytes at day 2,[26] which suggests that the reduction in infectivity was due to sex ratio distortion or a sterilising effect, or both.[4] In the current trial, we observed a 100% reduction in mosquito infection rate by day 5 for artemether–lumefantrine among individuals infectious to mosquitoes before treatment. However, there was an incomplete effect on gametocytes in two participants: one who was infectious on day 5 but not at baseline or on day 2, and another who was infectious at baseline and on day 5, but not on day 2. These instances of post-treatment infectivity might reflect differences in drug exposure of immature gametocytes that are sequestered in the bone marrow or spleen at the start of treatment but subsequently appear in circulation, or simply insufficient treatment of circulating mature gametocytes to prevent all transmission. It is therefore important that these observations are considered alongside our primary outcomes, and that further transmission studies are conducted to elucidate the gametocytocidal activity of artemether–lumefantrine against gametocytes at all stages of development, especially given the growing concerns of artemisinin resistance.

The potent transmission-blocking effect of artemether–lumefantrine contrasts with that of pyronaridine–artesunate and dihydroartemisinin–piperaquine, for which significant reductions in mosquito infection rate were only seen from 10 days and 7–14 days after treatment, respectively, when tested in the same facilities.[17,18] For individuals treated with sulfadoxine–pyrimethamine plus amodiaquine, considerable and prolonged post-treatment

transmission was observed. These data align with previous observations that show no change in transmission potential in the week following sulfadoxine–pyrimethamine plus amodiaquine treatment.[14] Male and female gametocyte density declined initially and then increased slightly at day 5; these transient increases in gametocyte density following sulfadoxine–pyrimethamine plus amodiaquine have been observed previously.[7] Here, ten (50%) of 20 individuals had more gametocytes at day 5 than at day 2 (in contrast to 5·3–15·8% in other treatment groups), which corresponded with increased infectiousness over the same time frame. Unlike in the artemether–lumefantrine groups and tafenoquine group, treatment with sulfadoxine–pyrimethamine plus amodiaquine did not cause any distortion in sex ratio. Although gametocyte prevalence in this group remained at 90% or more throughout the study, gametocyte densities declined in the majority of the participants to low, probably untransmissible levels by 21 days after treatment.[26]

As expected, the addition of one low dose of tafenoquine to sulfadoxine–pyrimethamine plus amodiaquine resulted in significant transmission-blocking activity among study participants. These results are consistent with previous studies in this setting, where the same low dose of tafenoquine (1·66 mg/kg) added to dihydroartemisinin–piperaquine led to complete transmission blockage at 7 days after treatment.[17] Recent studies have shown suboptimal *P vivax* relapse rates after tafenoquine radical cure when co-administered with dihydroartemisinin–piperaquine.[27] The reasons for this are unknown, but the combination appears effective for *P falciparum* transmission blockage, albeit with a delayed effect compared with primaquine.[17] The current study, observing similar timing of the transmission-blocking effect when combined with a non-ACT, argues against putative artemisinin-specific antagonism or interaction as the cause of the delayed effect.[27] In a controlled human malaria infection trial, the transmission-blocking efficacy of a single 50 mg dose of tafenoquine showed modest reduction of mosquito infection rate at day 4 of 35% and 81% by day 7.[28] This dose is approximately half of that given in the current trial (1·66 mg/kg), which resulted in a median reduction in mosquito infection rate of 100% (IQR 100–100), while participant infectivity dropped from 14 (70%) to two (10%) of 20.[17]

A few limitations in our study warrant consideration. First, there are a large number of secondary analyses, and although effect sizes are large, caution should be taken when interpreting them due to issues of multiple testing. Second, the primary outcome of the current trial was *P falciparum* transmission,

which required recruitment of high-density gametocyte carriers. With the assessment of infectivity before and after treatment, our findings are highly informative of the transmission-reducing activity of the tested drugs among highly infectious individuals. However, different study populations are required for assessments of community-level benefits of antimalarials combined with 8-aminoquinolines when given according to WHO guidelines (ie, at clinical presentation) or in mass treatment campaigns, including seasonal malaria chemoprevention. Third, our finding that a single low dose of primaquine appears to have little added benefit to transmission reduction when given with artemether–lumefantrine needs to be considered in light of possible use scenarios. Alternative ACTs with larger post-treatment transmission potential, such as dihydroartemisinin-piperaquine, are typically used in community treatment campaigns and the relevance of primaquine is likely to be larger. Moreover, gametocytes in *PfKelch13* mutant infections might preferentially survive artemisinin exposure and infect mosquitoes.[29] The addition of a non-artemisinin gametocytocide (eg, primaquine or tafenoquine) to standard treatment might be a valuable tool to limit the spread of artemisinin partial resistance in Africa; the WHO malaria policy and advisory group suggested the broader adoption of single low-dose primaquine in countries where partial resistance has been detected.[12] Finally, although tafenoquine is likely to have a comparatively prolonged transmission-blocking effect, its wider adoption is limited by concerns about potentially sustained haemolysis in G6PD-deficient individuals. Testing for G6PD deficiency is required before administration of tafenoquine for its current indications in treatment of *P vivax* (ie, 200 mg/day for 3 days or a single 300 mg dose).[30] This study enrolled only individuals with normal G6PD enzyme function, to ensure comparability between groups. We observed no haematological, grade 3, or serious adverse events in any treatment group, and transient reductions in haemoglobin density that were not significantly different between groups with and without 8-aminoquinonlines. Our results reinforce the previous observations that single low doses of primaquine and tafenoquine are safe and well tolerated but longitudinal safety data for tafenoquine in G6PD-deficient individuals are needed.

In conclusion, our findings show that artemether–lumefantrine is able to prevent nearly all mosquito infections, even without primaquine, while maintaining a good safety profile. Furthermore, we observed considerable post-treatment transmission following the use of sulfadoxine–pyrimethamine plus amodiaquine. Hence, the addition of a transmission-blocking drug might be useful in maximising the community benefit of seasonal malaria chemoprevention.

Contributors

WS, AM, MJS, TB, CD, and AD conceived the study and developed the study protocol. WS, AM, MJS, KS, YSi, SMN, AS, OMD, MD, SOM, YSa, SK, YD, SFT, AD, TB, CD, and AD implemented the trial. KL performed molecular analyses. WS, MJS, and AM verified the raw data. MJS, WS and JB analysed the data. WS, AM, MJS, RtH, JB, TB, CD, and AD wrote the first draft of the manuscript. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication. All authors read and approved the final manuscript.

Data sharing

The data from this trial are accessible on the Clinical Epidemiology Database Resources website under study name NECTAR3.

Declaration of interests

All authors declare no competing interests.

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References

1. Balikagala B, Fukuda N, Ikeda M, et al. Evidence of artemisinin-resistant malaria in Africa. N Engl J Med 2021; 385: 1163-71.
2. WHO. Global malaria programme: artemisinin resistance and artemisinin-based combination therapy efficacy: status report. https://www.who.int/docs/default-source/documents/publications/gmp/who-cds-gmp-2018-26-eng.pdf (accessed March 14, 2024).
3. Stepniewska K, Allen EN, Humphreys GS, et al. Safety of single-dose primaquine as a Plasmodium falciparum gametocytocide: a systematic review and meta-analysis of individual patient data. BMC Med 2022; 20: 350.
4. Bradley J, Soumaré HM, Mahamar A, et al. Transmission-blocking effects of primaquine and methylene blue suggest Plasmodium falciparum gametocyte sterilization rather than effects on sex ratio. Clin Infect Dis 2019; 69: 1436-39.
5. WHO. Global malaria programme: country antimalarial drug policies (Global AMDP database). https://www.who.int/teams/global-malaria-programme/case-management/treatment/country-antimalarial-drug-policies-by-who-regions/country-antimalarial-drug-policies-in-africa (accessed June 21, 2023).
6. Sawa P, Shekalaghe SA, Drakeley CJ, et al. Malaria transmission after artemether-lumefantrine and dihydroartemisinin-piperaquine: a randomized trial. J Infect Dis 2013; 207: 1637-45.
7. Bousema JT, Schneider P, Gouagna LC, et al. Moderate effect of artemisinin-based combination therapy on transmission of Plasmodium falciparum. J Infect Dis 2006; 193: 1151-59.
8. Mwaiswelo RO, Ngasala B, Msolo D, et al. A single low dose of primaquine is safe and sufficient to reduce transmission of Plasmodium falciparum gametocytes regardless of cytochrome P450 2D6 enzyme activity in Bagamoyo district, Tanzania. Malar J 2022; 21: 84.
9. Gonçalves BP, Tiono AB, Ouédraogo A, et al. Single low dose primaquine to reduce gametocyte carriage and Plasmodium falciparum transmission after artemether-lumefantrine in children with asymptomatic infection: a randomised, double-blind, placebo-controlled trial. BMC Med 2016; 14: 40.
10. Sutherland CJ, Ord R, Dunyo S, et al. Reduction of malaria transmission to Anopheles mosquitoes with a six-dose regimen of co-artemether. PLoS Med 2005; 2: e92.
11. WHO. Global malaria programme: policy brief on single-dose primaquine as gametocytocide in Plasmodium falciparum malaria. https://cdn.who.int/media/docs/default-source/documents/publications/gmp/policy-brief-on-single-dose-primaquine-as-a-gametocytocide-in-plasmodium-falciparum-malaria.pdf (accessed March 14, 2024).
12. WHO Malaria Policy Advisory Group. WHO Malaria Policy Advisory Group (MPAG) meeting report. https://iris.who.int/bitstream/handle/10665/368391/9789240074385-eng.pdf (accessed June 21, 2023).
13. Dicko A, Brown JM, Diawara H, et al. Primaquine to reduce transmission of Plasmodium falciparum malaria in Mali: a single-blind, dose-ranging, adaptive randomised phase 2 trial. Lancet Infect Dis 2016; 16: 674-84.
14. Dicko A, Roh ME, Diawara H, et al. Efficacy and safety of primaquine and methylene blue for prevention of Plasmodium falciparum transmission in Mali: a phase 2, single-blind, randomised controlled trial. Lancet Infect Dis 2018; 18: 627-39.

15. Bastiaens GJH, Tiono AB, Okebe J, et al. Safety of single low-dose primaquine in glucose-6-phosphate dehydrogenase deficient falciparum-infected African males: two open-label, randomized, safety trials. PLoS One 2018; 13: e0190272.
16. Hounkpatin AB, Kreidenweiss A, Held J. Clinical utility of tafenoquine in the prevention of relapse of Plasmodium vivax malaria: a review on the mode of action and emerging trial data. Infect Drug Resist 2019; 12: 553-70.
17. Stone W, Mahamar A, Smit MJ, et al. Single low-dose tafenoquine combined with dihydroartemisinin-piperaquine to reduce Plasmodium falciparum transmission in Ouelessebougou, Mali: a phase 2, single-blind, randomised clinical trial. Lancet Microbe 2022; 3: e336-e347.
18. Stone W, Mahamar A, Sanogo K, et al. Pyronaridine-artesunate or dihydroartemisinin-piperaquine combined with single low-dose primaquine to prevent Plasmodium falciparum malaria transmission in Ouélessébougou, Mali: a four-arm, single-blind, phase 2/3, randomised trial. Lancet Microbe 2022; 3: e41-e51.
19. WHO. Seasonal malaria chemoprevention with sulfadoxine–pyrimethamine plus amodiaquine in children: a field guide. https://www.afro.who.int/sites/default/files/2017-06/9789241504737_eng.pdf (accessed March 14, 2024).
20. WHO. Seasonal malaria chemoprevention with sulfadoxine–pyrimethamine plus amodiaquine in children: a field guide. https://www.who.int/publications/i/item/9789240073692 (accessed March 14, 2024).
21. Meerstein-Kessel L, Andolina C, Carrio E, et al. A multiplex assay for the sensitive detection and quantification of male and female Plasmodium falciparum gametocytes. Malar J 2018; 17: 441.
22. Bousema T, Okell L, Shekalaghe S, et al. Revisiting the circulation time of Plasmodium falciparum gametocytes: molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. Malar J 2010; 9: 136.
23. Feachem RGA, Chen I, Akbari O, et al. Malaria eradication within a generation: ambitious, achievable, and necessary. Lancet 2019; 394: 1056-112.
24. WWARN Gametocyte Study Group. Gametocyte carriage in uncomplicated Plasmodium falciparum malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data. BMC Med 2016; 14: 79.
25. Roth JM, Sawa P, Omweri G, et al. Plasmodium falciparum gametocyte dynamics after pyronaridine-artesunate or artemether-lumefantrine treatment. Malar J 2018; 17: 223.
26. Meibalan E, Barry A, Gibbins MP, et al. Plasmodium falciparum gametocyte density and infectivity in peripheral blood and skin tissue of naturally infected parasite carriers in Burkina Faso. J Infect Dis 2021; 223: 1822-30.
27. Sutanto I, Soebandrio A, Ekawati LL, et al. Tafenoquine co-administered with dihydroartemisinin-piperaquine for the radical cure of Plasmodium vivax malaria (INSPECTOR): a randomised, placebo-controlled, efficacy and safety study. Lancet Infect Dis 2023; 23: 1153-63.
28. Webster R, Mitchell H, Peters JM, et al. Transmission blocking activity of low-dose tafenoquine in healthy volunteers experimentally infected with Plasmodium falciparum. Clin Infect Dis 2023; 76: 506-12.
29. Witmer K, Dahalan FA, Delves MJ, et al. Transmission of artemisinin-resistant malaria parasites to mosquitoes under antimalarial drug pressure. Antimicrob Agents Chemother 2020; 65: e00898-20.
30. US Centers for Disease Control and Prevention. Tafenoquine approved for malaria prophylaxis and treatment. https://wwwnc.cdc.gov/travel/news-announcements/tafenoquine-malaria-prophylaxis-and-treatment (accessed July 27, 2023).

Supplementary Material

Please scan the QR code below to access the supplementary material for Chapter 3A:

CHAPTER 3B

Life cycle assessment of a clinical malaria trial in Mali reveals large environmental impacts of electricity consumption and international travel

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Abstract

Climate change may be the single largest threat facing humanity and ecosystems, necessitating reductions in carbon emissions across all sectors, including healthcare and academia. With the aim of informing and supporting sustainable research practices, we performed a life cycle assessment of a clinical malaria trial conducted in Mali. The trial involved 80 malaria-infected participants in Ouélessébougou who were treated with antimalarials and monitored to determine clinical and transmission-blocking efficacy. Data on consumables, transportation, travel, and electricity use were collected in Mali and the Netherlands, where additional laboratory analyses and sample storage occurred. Data were analysed using the ReCiPe 2016 method for midpoint impact assessment. The trial involved 3 intercontinental shipments of materials and samples, 59,900 km of travel by research staff, and ~55 kg of plastic consumables. Trial conduct and reporting resulted in approximately 20.5 metric tons of CO_2-equivalent (CO_2e) emissions. Major carbon contributors were international travel (50%), electricity in Mali (28%), and air-transportation of materials (14%). Laboratory consumables, while contributing up to 20% of the trial's impact on land and water use, were less important sources of emissions (2% of CO_2e). The formation of fine particulate matter was another important contributor to human health damage, which was mainly attributed to electricity in Mali. Main contributors to ecosystem damage were carbon emissions, terrestrial acidification and ozone formation, with electricity in Mali and international travel as the two major contributors. With an eye on energy efficiency and sustainability, we observed no loss in stability of parasite genetic material (mRNA) in protective buffers when stored for 12 months at -20°C, compared to conventional -70°C. Switching to energy-efficient equipment settings could reduce electricity consumption of equipment by over 30%. Implementing solar panels could reduce overall CO_2e emissions substantially. Immediate CO_2e reductions can further be achieved through online conference attendance and alternative sample transportation; the latter would allow 10% CO_2e emission reduction. These results form a starting point for improving the environmental sustainability of clinical trials in Africa.

Author summary

Our study is the first to examine the environmental impact of a clinical trial conducted in Africa where we estimated carbon emissions and other environmental impacts. We found that the trial, which involved treating

and monitoring malaria-infected participants, resulted in 20.5 tons of CO_2-equivalent emissions. The largest contributors were travel (50%) and electricity use in Mali (28%). Other important contributors to environmental damage were the formation of fine particulate matter, terrestrial acidification and ozone formation. For these impact factors, electricity use and international travel were also the two primary drivers. While laboratory consumables had a high impact on land and water use (up to 20%), their impact on the carbon footprint and other impact factors was minimal. In addition to measuring the trials environmental impact, we explored practical ways to reduce this impact, such as using energy-efficient equipment, storing samples at higher temperatures, and finding alternative ways to transport materials. Our work highlights the importance of making clinical research more sustainable and shows how similar studies can lower their environmental footprint. By reducing air travel and switching to renewable energy sources, future trials can significantly reduce their CO_2e emissions, fine particulate matter formation, terrestrial acidification and ozone formation. These findings offer guidance for researchers and organizations to adopt environmentally sustainable practices in their trials.

Introduction

Climate change is considered one of the largest – potentially the single largest – threats to humanity and global health.[1,2] Climate change affects many social and environmental determinants of health, including the availability of clean air, safe drinking water, sufficient food, and secure shelter. The health burden of climate change is disproportionally carried by poorer countries.[3-5] Whilst the African continent is responsible for less than 4% of global carbon emissions since the industrial revolution,[6] its burden in terms of disability adjusted life years lost due to climate change is estimated to be over 100-fold larger than that of high-income countries.[4] One of the potential direct consequences of global warming on human health is the aggravation of human infectious diseases.[7] Health systems must adapt to the reality of climate change, but may also play a role in mitigation since they are relevant contributors to global warming and other ecological calamities. The healthcare sector is estimated to be responsible for 1–5% of the total ecological footprint of human activities, with considerable variation between countries.[8] The Dutch healthcare sector accounts for 7.3% of the nation's carbon footprint and contributes to a broad set of environmental impact categories beyond climate change.[9] Life cycle

assessment (LCA) is a footprint analysis at product or service level that covers multiple impact categories. Trade-offs may occur between different impact categories. When considering environmental sustainability, global warming receives most attention but other factors like fine particulate matter formation, acidification, ozone depletion, freshwater depletion, and land use are also major concerns for the earth's ecosystems and, as a consequence, human health.[10] Here, we conducted an LCA of a clinical trial involving different antimalarial treatment regimens. Our work aimed to better understand the interplay between various environmental impact categories and make informed decisions that promote sustainable research practices.

Materials and methods

Overview clinical trial

During a phase 2 clinical trial investigating the efficacy of different antimalarial treatment regimens and their impact on malaria transmission to mosquitoes, a total of eighty participants underwent 28-days of follow-up.[11] Trial outcome measures encompassed safety, including clinical, hematological, and biochemical analyses, as well as microscopical and molecular quantification of *Plasmodium* parasites stages, and mosquito feeding assays. The study was conducted in Ouélessébougou, Mali, as part of an international collaboration between Malian, Dutch, and UK research institutes. During the conduct of the study in Mali, the ensuing analyses in the Netherlands, and the dissemination of study results at a conference in the United States, data on the use of consumables, electricity, transport, and travel were collected.

Participants provided written informed consent (≥18 years) or assent with written parental consent (for participants aged 10-17 years). Ethical approval for the clinical trial was granted by the Ethics Committee of the Faculty of Medicine, Pharmacy, and Dentistry of the University of Science, Techniques, and Technologies of Bamako (Bamako, Mali) (No2021/189/CE/USTTB), and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (London, United Kingdom) (LSHTM Ethics Ref: 26257).

Study medication

In preparation of the study, 1290 participants were screened for eligibility criteria in 9 villages near Ouélessébougou. Following enrolment into the clinical trial, 80 participants received the study medication with (combinations

of) 20/120 mg or 80/480 mg artemether/lumefantrine tablets (Coartem, Novartis), 30 mg primaquine tablets (A-PQ 30; ACE Pharmaceuticals), 500/50 mg sulfadoxine/pyrimethamine tablets (Guilin Pharmaceutical), 150 mg amodiaquine tablets (Guilin Pharmaceutical), or 100 mg tafenoquine (Arakoda, 60 Degrees Pharmaceuticals) tablets. 77 out of 80 participants completed the scheduled eight follow-up visits; three participants withdrew consent after the first follow-up visit.

Materials and their transportation and disposal

In addition to locally sourced materials (including microtainers, slide boxes, lancets), two shipments and two parcels with additional consumables (including needles, tubes, pipet tips, gloves, pregnancy tests, labels, microscope slides) were shipped from the Netherlands and the UK to the study site (Fig 1). One of the parcels was routed through 6 different countries prior to delivery in Mali. Study medication that could not be sourced in Mali came from the Netherlands (Fig 1). In addition to this transportation to Mali, there was a single shipment of 34 standard 13 x 13 x 5 cm freezer boxes with study samples on dry ice after completion of the trial. For this shipment, 290 kg of dry ice was shipped from France to Mali and then on to the Netherlands to transport the study samples in frozen condition. All potentially infectious materials were incinerated at 850 – 1000°C.

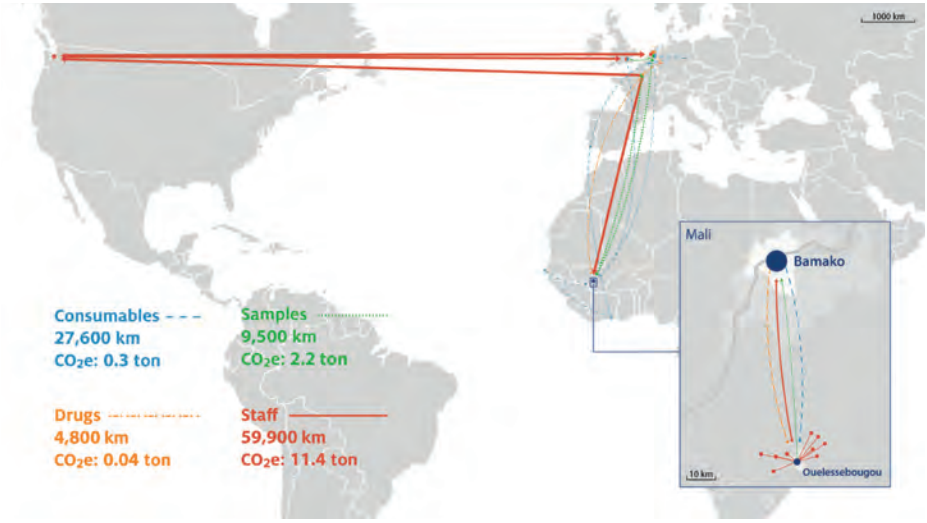

Figure 1. Travel and transport of consumables, samples, drugs, and staff. Distance is based on actual routes, emissions are based on the LCA outcomes and direct emissions (e.g., CO_2), as well as emission of chemical species that alter radiatively active substances or trigger generation of aerosol particles. Staff travel includes air travel and travel by road in Mali.

Laboratory analyses

Stability of nucleic acids at higher storage conditions

To determine whether it is possible to avoid the use of energy-intensive ultra-low temperature freezers,[12] we tested the stability of parasite messenger RNA, important for secondary outcomes of the trial, at different conditions. Freezers with extracted and unextracted RNA are set to -70°C (instead of conventional -80°C) at Radboudumc for sustainability reasons. Serial dilutions of the parasite stage that is of prime interest for the study (gametocytes, *Plasmodium falciparum* NF54 line) were cultured, diluted in whole-blood and preserved in RNAprotect Cell Reagent (Qiagen, Germany) to stabilize parasite mRNA for later gametocyte quantification. After mixing, samples were stored in either a -70°C or a -20°C freezer for 2 weeks, 3, 6, or 12 months after which total RNA was extracted using a MagNAPure automated extractor (Total Nucleic Acid Isolation Kit-High Performance; Roche Applied Science, Indianapolis, IN, USA). Gametocytes were detected by CCp4/PfMGET RT-qPCR;[13] CT-values that are indicative of mRNA abundance were presented for different storage conditions.

Temperature stability of a container for sample transport

Anticipating RNA stability at -20°C, we considered transportation of samples in a temperature controlled box (Crēdo Cube, Peli BioThermal, Torrance, United States) that allows maintaining temperature at or below this temperature. We tested temperature stability using a LIBERO CL V9.14 probe that was stored in the cube after its elements had been charged for 24 hours in a freezer set at -20°C or -70°C.

Life cycle assessment (LCA)

An LCA was conducted to estimate the environmental impact of the trial and identify areas where measures for reducing environmental impacts might be applied most effectively. The LCA encompassed the entire life cycle of the product system including creation, use, and disposal of a product. This includes everything from extraction of raw materials to manufacturing, distribution, use, and finally the management of waste. It covers all stages of a product's life cycle, from its "cradle" to its "grave".[14] The functional unit of this study is defined as the conduction of the entire clinical trial over a duration of three months in Mali followed by one additional month of analyses in the Netherlands. The inventory of material flows for the current study was categorized into eleven main groups: travel of employees via air, travel of employees and participants

via road, transport of materials via air, transport of materials via road, electricity in Mali, electricity in the Netherlands, medication, safety analysis, mosquito infection analyses, molecular analyses, and others. The last four groups primarily consist of consumables. Due to the absence of life cycle inventory data on the production of the antimalarials used in this trial, data on CO_2e emissions from the production of other pharmaceuticals that are also produced on a large scale, namely vancomycin and tenofovir, were used instead.[15] We averaged the impacts of these two medications as estimated impact of the used malaria medication. For the packaging material, we modeled polyvinylchloride and aluminum blister packaging. For all other categories, we collected data on raw material extraction, product manufacturing, transportation, usage, and end-of-life stage. Data were gathered through a combination of direct observations by the study team, literature review, public databases (ecoinvent 3.9, Switzerland and healthcareLCA.com), and communication with manufacturers. Product consumption was based on the supplies purchased for the different study procedures and direct observations during study conduct (e.g., electricity consumption, transport of materials, and travel of participants and staff). Materials and their weight, quantity, and material composition were identified. A full list of measurements and assumptions are given in the appendix. Electricity usage at the research site in Mali was estimated by distributing the total electricity use of the field station (6862 – 7763 kWh usage per month) according to the number of studies being conducted during the study period (1st of October - 31st of December 2021). The electricity mix in Mali was assumed to be ~60% based on fossil fuels.[16] For electricity use in the Netherlands, we used a similar approach where electricity usage of the 7-floor research infrastructure in the month the analyses were performed (262.965 kWh usage for February 2022) was allocated to the project based on the size of the dedicated lab (20 m^2) relative to lab space in the entire building (3803 m^2). Radboudumc uses 100% renewable energy, supported by an institutional Guarantee of Origin for consumed electricity and the construction of an onshore windfarm by Radboudumc and Radboud University with ten >3MW wind turbines. To further gauge electricity use of equipment that was specifically used for the current study, we measured electricity use in kWh using the a plug-in electricity meter (Energie Meter Mini; EcoSavers, the Netherlands); also these data are provided in the appendix. For the current study, certain processes were considered non-contributory as they extended beyond the system's scope and expected to have a minimal contribution to the overall study impact. For example, the production of capital goods, such as routine lab equipment, and lab/hospital infrastructure were excluded from the system boundary (Fig 2).

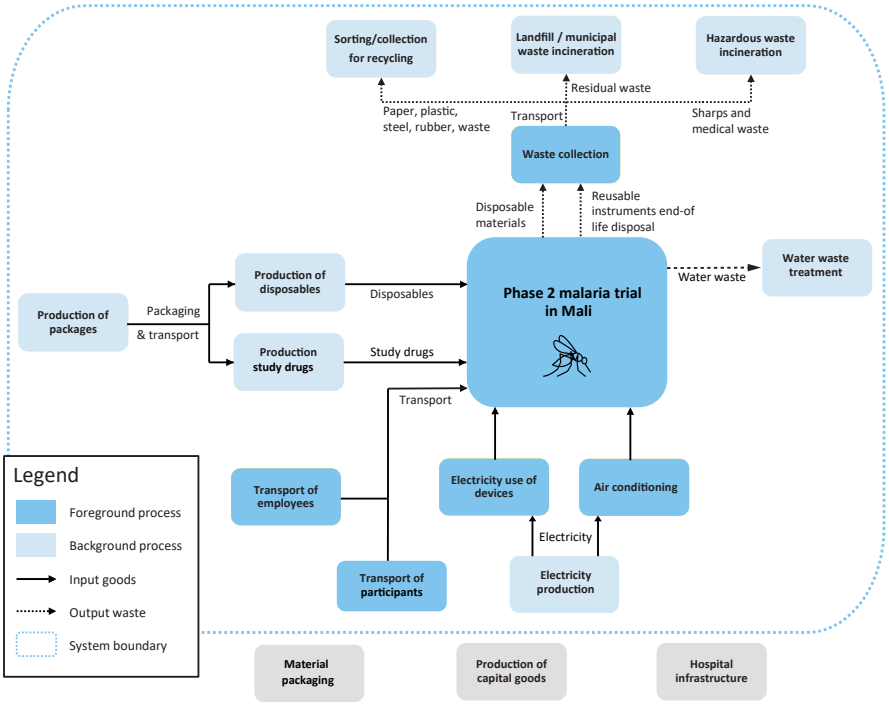

Figure 2. The system boundary. System boundary of what was included (within the blue dotted boundary) and excluded in the life cycle assessment.

Life cycle impact assessment and sensitivity analysis

The environmental impact scores in the Life Cycle Impact Assessment (LCIA) were computed using the ReCiPe 2016 method,[17] which models the impact of (components of) products on environmental midpoints and endpoints. This method encompasses a total of 18 midpoint indicators/characterization factors that can be consolidated into three endpoint indicators: damage to human health, damage to ecosystems, and damage to resource availability. In the current study, the impact on global warming, land, and water usage was reported in detail. The data were modeled using Sima Pro 9 LCA software from PRé Consultants in Amersfoort, the Netherlands. To estimate the carbon emissions associated with air travel, we used established methodologies that use mean emission factors per km and per passenger of three independent sources and take into account direct emissions as well as indirect emissions resulting from chemical species that alter radiatively active substances and the generation of aerosol particles.[18] We conducted an uncertainty analysis using the pedigree matrix and the Monte Carlo algorithm with 1,000 runs. To confirm the robustness of the findings, we conducted a sensitivity analysis

by examining the impact of various assumptions, database selections, and analytical methods on the identified key areas.[19] Within this sensitivity analysis, we focused on the comparison of different African energy mixes and the assumptions made for the impact calculations of medication. We also assumed an alternative method to estimate CO_2e emissions associated with air travel (see appendix).[17]

Results

Below, we provide a narrative of several key factors that influence the environmental impact of the clinical trial. A comprehensive list of (raw) material use, electricity consumption and travel/movement of goods for the study in Mali and associated activities in the Netherlands and the United Kingdom (UK) is presented in the appendix.

Consumables and electricity use

During sampling at screening, enrolment, and follow-up visits in Mali, 231 stainless steel lancets, 1607 needles, 1607 glass microscope slides, 4821 vacutainer tubes, and 3418 microtainer collection or storage tubes were used. Treatment involved 420 tablets artemether/lumefantrine, 20 tablets primaquine, 291 tablets sulfadoxine/pyrimethamine, 361 tablets amodiaquine, and 24 tablets tafenoquine.

In running the trial facilities in Ouélessébougou, a total of 7,200 kWh of electricity was used. Freezers, laboratory equipment, and air conditioners were important contributors to the electricity consumption in Mali (Fig 3). In the Netherlands, a total of 1,383 kWh of electricity was used. Laboratory activities included total nucleic acids extraction using an automated extractor (17 runs of 1.6 kWh) and a total of 2,040 plastic tips (0.8 kg of virgin polypropylene). Following extraction, a single multiplex PCR was performed (7 runs of 0.55 kWh), using a total of 721 plastic tips.

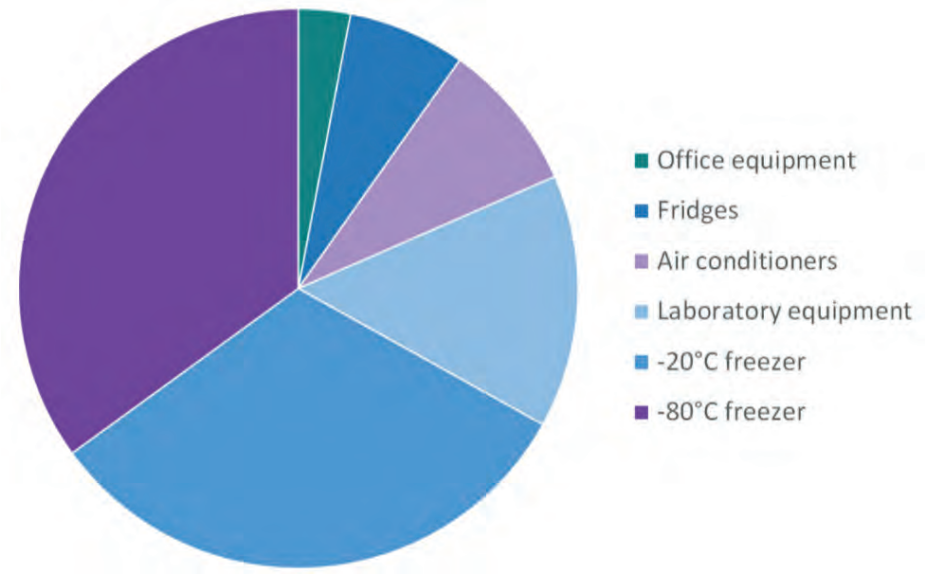

Figure 3. Sources of electricity consumption in Mali during the study period. Laboratory equipment included centrifuges, equipment for biochemistry and haematology, incubators and waterbaths but not freezers and fridges that are presented separately. Note that freezers in Mali were set at -80°C whilst in the Netherlands they were set at -70°C. Office equipment includes computers and printers.

To explore more energy efficient study procedures, mRNA samples in whole blood were stored for up to one year at -20°C and -70°C in protective buffer. The two storage temperatures gave comparable signal levels for mRNA quantification (Fig 4A). Whilst there were indications for increased CT-values after 6 months of storage for one of the mRNA targets (PfMGET), indicating lower mRNA concentrations, this pattern was similar for both storage temperatures and not observed for the other mRNA target (CCp4). The temperature log in the temperature controlled box showed that when the elements were charged at -20°C, the temperature remained below -20°C for 20 hours; when elements were charged at -70°C for 24 hours, the temperature remained below -20°C for over 4 days (108 hours) (Fig 4B).

Travel-associated environmental impacts

During the conduct of the study, the Malian team utilized one Toyota Land Cruiser 105 and two Toyota Hilux pick-ups to travel to the 9 different villages where study participants resided. In total, they covered over 3,300 km to conduct follow-up visits. During these journeys, approximately 334 litres of diesel were used. Emissions associated with the use of vehicles were

approximately 1,150 kg of CO_2e. After completion of primary data collection, three team members travelled to a conference in Seattle, the United States, to present study results to an international audience and discuss study progress with funders. For this conference, team members travelled approximately 56,600 km and, in doing so, emitted an estimated 10,200 kg of CO_2e emissions. Because of difficulties in obtaining a visa, a fourth person presented the main study results during online participation. This online participation was associated with approximately 0.33 kg of CO_2e emissions for the entire 4-day conference.[20]

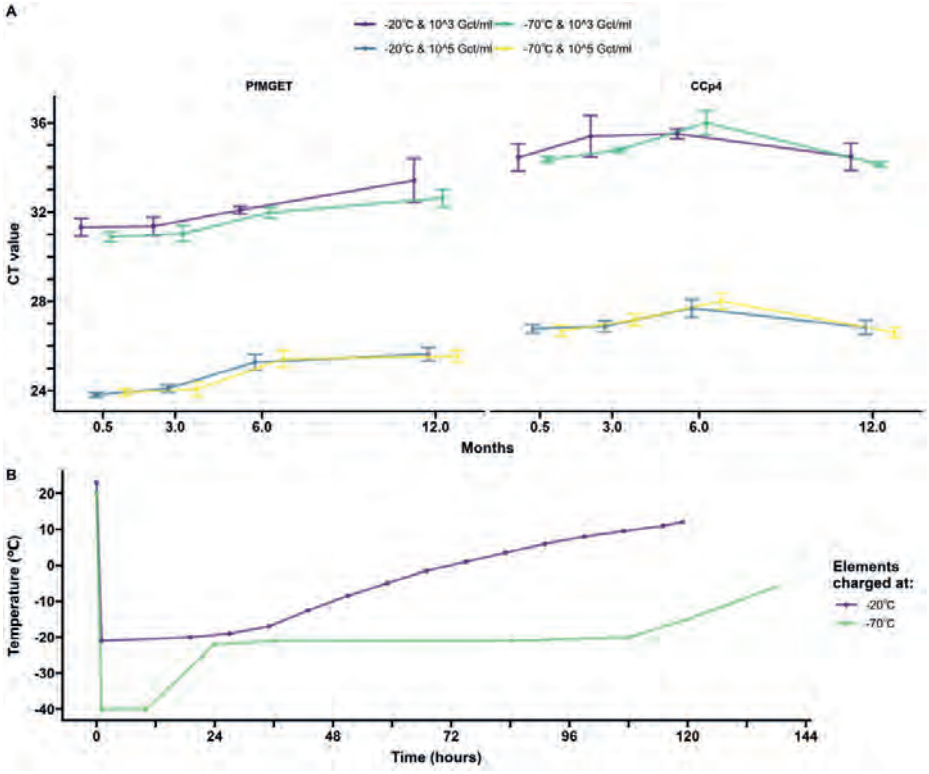

Figure 4. (A) RNA stability and (B) temperature stability of a temperature controlled box. (A) RNA stability was tested by quantifying gametocytes in *P. falciparum*-positive samples stored at -20°C and -70°C in protective buffers, based on the expression of the CCp4 (female gametocyte) and PfMGET (male gametocyte) genes using RT-qPCR. The average cycle threshold (CT) values for PfMGET and CCP4 transcripts at different temperatures are shown with error bars. (B) Elements of the temperature-controlled box were charged at different temperatures (-20°C and -70°C), and the temperature in the box was monitored for 5-6 days.

Estimated impact of the trial on global warming, fine particulate matter formation, terrestrial acidification, ozone formation, water use, and land use

Total carbon emissions, as estimated by LCA, were approximately 20.5 metric tons of CO_2e. International travel accounted for approximately 50% of this total (Fig 5A); other important contributors were electricity consumption in Mali (28%) and air transportation of materials (14%). Laboratory consumables were considerable contributors to the ecological impact of the study in terms of land and water use impact (up to 20%) but only accounted for 2% of the total CO_2e emissions (Fig 5B-C).

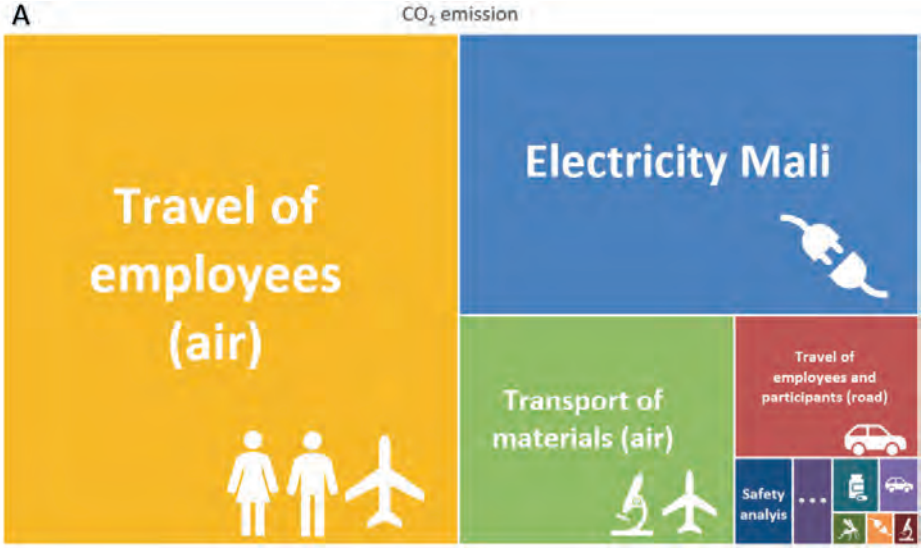

Figure 5A. Tree map of the distribution of CO2e emission of the clinical trial. Each rectangle represents a different component of the trial, with the size of the rectangle proportional to the CO_2e emission. The colours indicate the different components of the trial: yellow for air travel of employees (49.8%), blue for electricity in Mali (27.7%), light green for transport of materials via air (13.5%), red for travel of employees and participants by road (5.6%), dark blue for the safety analysis (1.1%), dark purple for others (0.7%), turquoise for medication (0.5%), light purple for transport of materials via road (0.5%), dark green for mosquito infection analyses (0.2%), orange for electricity in the Netherlands (0.2%), and maroon red for molecular analyses (0.2%).

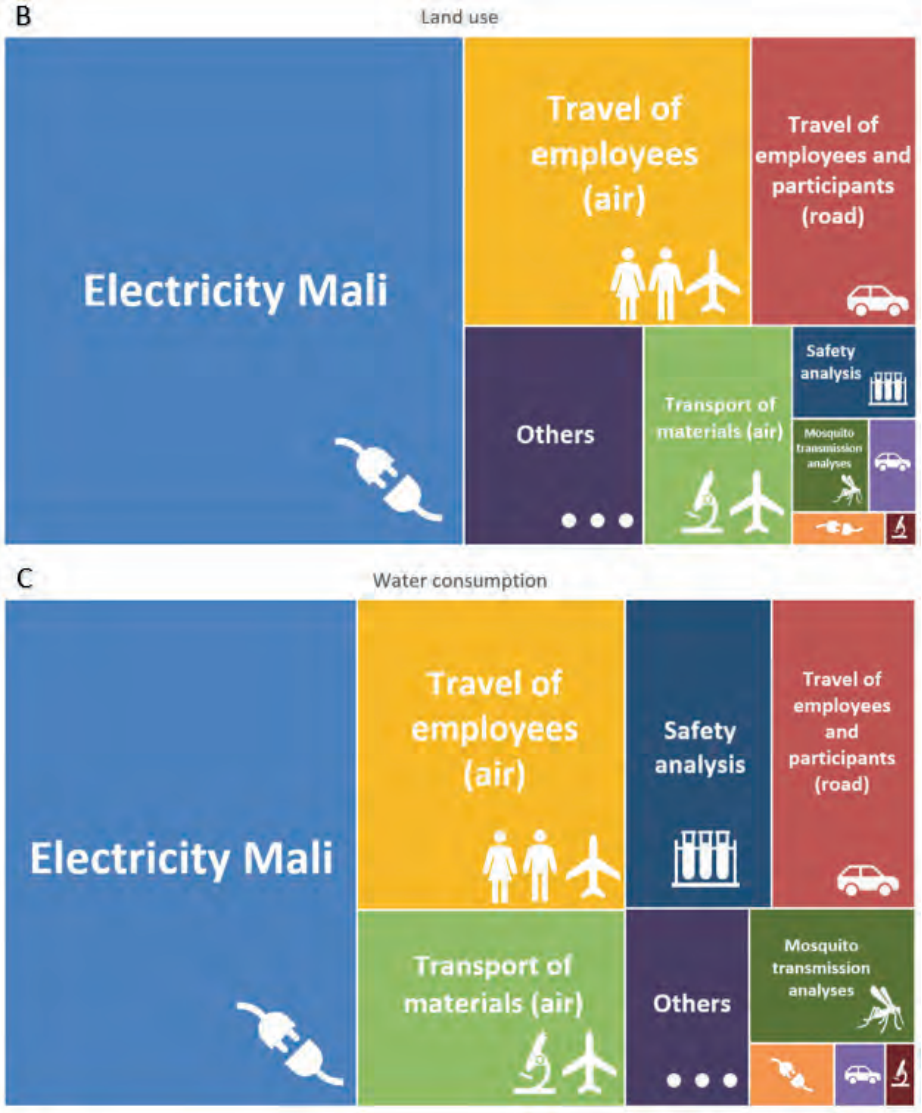

Figure 5B-C. Tree map of the impact categories 'Land use' and 'Water consumption' of the clinical trial. The colours indicate the different components of the trial: blue for electricity in Mali (50.5% and 38.7% in land use and water consumption, respectively), yellow for air travel of employees (17.9% and 18.0%), red for travel of employees and participants by road (10.3% and 9.6%), dark purple for others (8.5% and 5.3%), light green for transport of materials via air (7.0% and 11.4%), dark blue for the safety analysis (2.5% and 9.8%), dark green for mosquito infection analyses (1.6% and 4.9%), light purple for transport of materials via road (0.9% and 0.7%), orange for electricity in the Netherlands (0.7% and 1.2%), maroon red for molecular analyses (0.2% and 0.4%), and turquoise for medication (0.0% and 0.0%).

Another midpoint significantly contributing to human health damage was fine particulate matter formation with 21 kg PM2.5eq (Fig 6). Electricity in Mali (49%) and international travel (31%) were also the main contributors within this impact category. When investigating the impact on ecosystem damage, next to global warming, also terrestrial acidification (64 kg SO_2 eq) and ozone formation (75 kg NO_x eq) were major contributors, again mostly attributed to electricity in Mali (34 kg of SO_2 eq and 19 kg of NO_x eq) and international travel (19 kg of SO_2 eq and 39 kg of NO_x eq). Other impact factors, like water use (35,000 litre) and land use (286 m^2a crop eq) had a relatively small contribution to the overall environmental damage. The category contributing most to these impact factors was the use of laboratory consumables, mainly the production of disposables and soap.

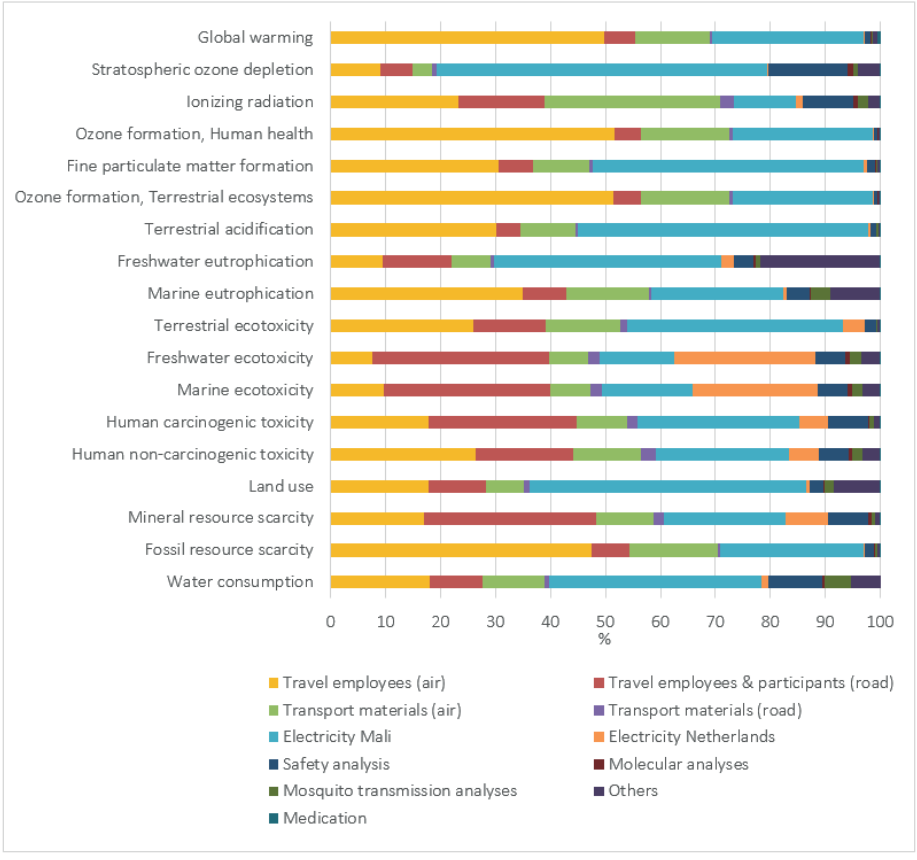

Figure 6. The contribution of each category of the trial to 18 midpoint environmental impact categories. For each category the total impact is set to 100% and the relative contribution of different activities and products is indicated in the same colour scheme that was used for Fig 5.

Sensitivity and uncertainty analysis

To assess robustness of findings, we performed a sensitivity analysis where we assumed a different energy mix for electricity generation (the energy mix of Mali vs. neighbouring countries Guinea and Niger); this resulted in a variation in global warming from -12% to 11%. In the case of the energy mix of Niger, the total CO_2e emission increased from 20.5 to 22.8 metric tons, whereas the emission decreased to 18.0 metric tons if the energy mix of Guinea was used. However, these variations did not reveal different hotspots than the original analyses. Similarly, testing assumptions for medication did not significantly change the identified hotspots or results (see appendix). Depending on the choice of proxy for the medication calculation, the relative impact of medication varied between 0.1% and 1% of the total CO_2e emissions. Utilising the established ReCiPe 2016 method with ecoinvent 3.9 as background data source to calculate the CO_2e contribution from international air travel in the sensitivity analysis resulted in a lower estimate (7.9 metric tons) compared to the more conservative approach (10.2 metric tons), which also included indirect sources of CO_2e emissions, such as radiatively active substances and substances that trigger generation of aerosol particles. Despite the differences in calculations, the top two contributors to the trial's carbon footprint–electricity consumption in Mali and air travel–remain the same, regardless of the approach used. The 95% confidence interval for the global warming potential associated with the study was found to be between 16.1 and 22.0 metric tons CO_2e. Confidence intervals for other environmental impact categories are detailed in the appendix.

Discussion

Here, we present one of the first life cycle assessments of a clinical trial, examining the trial's ecological impact beyond its carbon footprint alone. Our analysis estimates the environmental impact of the clinical malaria trial and related activities to be approximately 20.5 metric tons of CO_2e. The main sources of CO_2e emissions were international travel (50%), electricity usage in Mali (28%), and air-transportation of materials (14%). Besides the carbon footprint, fine particulate matter formation, terrestrial acidification and ozone formation had a substantial overall impact on the environment (more than 90% of the total impact on human health and ecosystem damage). Notably, the major contributing categories to the latter impact factors did not differ from the categories contributing to the carbon footprint. Goods travelled a total of 41,900 km while study personnel travelled 3,300 km to and from study sites

and 56,600 km to a conference. We estimate that the study used a total of 55 kg of plastics and 5 kg of glass across participating centres.

Clinical trials are associated with considerable CO_2e emissions, notably through energy use at research premises, transportation, and (air) travel.[21] Our study identifies that international travel and electricity consumption in Mali collectively accounted for 78% of the trial's carbon footprint. Assessments of the ecological impact of clinical (research) activities on the African continent are very sparse and drivers of this impact may differ from other settings. In our study, we observed a large CO_2e contribution of international air travel. Of note, this study was conducted in a period when COVID-19 related travel restrictions were imposed and there was no international travel during the preparation and conduct of the clinical trial. Whilst the nature of the collaboration, being intercontinental, may have increased travel and shipment kilometres, the importance of transport as driver of emissions appears a consistent finding across LCA studies on clinical trials. For example, an LCA of a phase 1 clinical trial in Belgium involving 28 participants generated 17.65 tons of CO_2e, with the movement of participants and staff accounting for the majority of emissions (51%), followed by trial site utilities (16% of overall emissions).[22] A phase 2 international trial in the UK, Spain, and Australia, testing an investigational medicinal product with 48 participants, reported a carbon footprint of 72 tons CO_2e, with the majority of emissions coming from the clinical trials unit and staff travel.[23] However, main contributors do differ between trials. For instance, a phase 3 trial conducted in the UK with 1,962 patients for a non-investigational medicinal product, generated 89 tons CO_2e, largely due to the intramural assessments required for participants.[23] A retrospective assessment of the total carbon footprints of three multicentre phase 3 trials in North America, South America, Europe, and Asia estimated a total of 1,437 – 2,498 tons CO_2e per trial involving 668 – 4,744 participants and identified travel and shipment of samples and materials as important drivers of this footprint.[24] Additionally, there were considerable emissions attributed to the shipment and storage of samples for future use.[24] This means that also the 'hotspots' for adjustments to reduce the environmental impact can differ between studies.

Long-term storage of materials was not included in our analysis and is likely to have minimal impact considering the use of renewable energy in the country where the samples are stored. Storage does, however, allow for simple improvements in electricity consumption and freezer purchases.[24] We identified several other sources of emissions and material use that offer

opportunities for emission reductions. First of all, we demonstrated similar stability of samples when stored at -20°C as compared to -70°C for at least one year. This allows initial storage at -20°C and a more environmentally conscious method for transporting study samples (i.e., temperature controlled boxes compared to using air-transported dry ice). We confirmed that these temperature controlled boxes indeed maintain appropriate freezing conditions. Another easily effected change would be to reduce the amount of (air-)travel involved in trial conduct and dissemination. Large CO_2e reductions can clearly be achieved by limiting the number of team members attending international conferences in person and instead encouraging virtual conference attendance for some team members. Furthermore, maintaining the shift in academic expertise from north to south would also lessen the need for air travel. Whilst we consider in-person meetings important to sustain collaborations and offer career development opportunities, the frequency of intercontinental flights offers opportunities for emission reduction. Our sensitivity analysis demonstrates that the assumptions underlying the estimated CO_2e emissions of travel are relevant. Logically, calculations that include indirect sources of emissions due to the emission of chemical species that alter radiatively active substances or trigger generation of aerosol particles,[18,25] increase the estimated impact of air travel. However, even with the conservative ReCiPe 2016 method, air travel is a major source of CO_2e emissions. In addition to reducing air travel, sourcing plastics and reagents locally or regionally would be highly beneficial to reduce transport costs and emissions.

Several other sources of emission will likely pose a greater challenge. The importance of the local energy generation mix was demonstrated by the negligible (<1%) contribution of analyses and storage in the Netherlands that benefited from renewable energy. The stronger reliance on fossil fuels for electricity generation in Mali, had a major impact on emissions and is unlikely to change in the near future. Implementing solar panels in the Mali laboratory could have reduced CO_2e emissions by 28%.

In our trial, consumables contributed considerably to the study's ecological impact in terms of land and water use impact (up to 20%), but represented only a relatively minor fraction (2%) of the total CO_2e emissions. This underscores the complex interplay between components within the trial's life cycle and their respective contributions to environmental impact.

Whilst the assessment of environmental impacts other than carbon emissions is a relevant strength of our study, our study also has several limitations. Several environmental impacts will be setting and study dependent; our study in Africa with an international research team plausibly resulted in a larger contribution of air travel. Our study was also modest in size and short in duration; the period of data collection and laboratory analyses may be considerably longer for other phase II studies. We also made assumptions on the impacts of study medication based on the CO_2e emissions of other medications that are produced at similarly large scale. However, we were limited to including only the CO_2e emissions of the study medication, leaving out the other 17 factors, which represents a knowledge gap. Whilst our assumptions are unlikely to have affected the relative impact of different sources, it will have affected generalizability. Lastly, while we provide an assessment of the environmental impact of this specific trial, it is important to weigh this impact against the knowledge and health benefits that well-designed studies, which address relevant knowledge gaps, bring.

Our analysis also indicates that dissemination activities, particularly international travel for conferences, were a major contributor to the trial's overall environmental footprint. This highlights the need to consider the environmental implications of dissemination in future studies, particularly in relation to air travel. Approaches such as encouraging virtual conference participation and decentralizing academic expertise to reduce the necessity for travel could help reduce these impacts.

We conclude that the academic community has a role in exploring not just what we research but also how we research.[26] Global health research faces the complex task of addressing climate-driven health and health system challenges while at the same time reducing its own ecological impact. The healthcare industry can utilize research, data, and quantitative analysis tools to make informed environmental decisions for practice, as we demonstrate here at the scale of a single clinical trial. It is important to adopt sustainable and low carbon research practices that still deliver the scientific advances that society needs.[26]

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References

1. Mora C, Spirandelli D, Franklin EC, Lynham J, Kantar MB, Miles W, et al. Broad threat to humanity from cumulative climate hazards intensified by greenhouse gas emissions. Nature Clim Change 2018; 8(12): 1062–71.
2. World Health Organization. Climate change and health 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/climate-change-and-health
3. Burkart KG, Brauer M, Aravkin AY, Godwin WW, Hay SI, He J, et al. Estimating the cause-specific relative risks of non-optimal temperature on daily mortality: a two-part modelling approach applied to the Global Burden of Disease Study. Lancet 2021; 398(10301): 685–97.
4. Costello A, Abbas M, Allen A, Ball S, Bell S, Bellamy R, et al. Managing the health effects of climate change: Lancet and University College London Institute for Global Health Commission. Lancet 2009; 373(9676): 1693–733.
5. Gasparrini A, Guo Y, Sera F, Vicedo-Cabrera AM, Huber V, Tong S, et al. Projections of temperature-related excess mortality under climate change scenarios. Lancet Planet Health 2017; 1(9): e360–7.
6. Statista. Africa's share in global carbon dioxide (CO_2) emissions from 2000 to 2021 2023. Available from: https://www.statista.com/statistics/1287508/africa-share-in-global-co2-emissions/
7. Mora C, McKenzie T, Gaw IM, Dean JM, von Hammerstein H, Knudson TA, et al. Over half of known human pathogenic diseases can be aggravated by climate change. Nat Clim Chang 2022; 12(9): 869–75.
8. Lenzen M, Malik A, Li M, Fry J, Weisz H, Pichler P-P, et al. The environmental footprint of health care: a global assessment. Lancet Planet Health 2020; 4(7): e271–9.
9. Steenmeijer MA, Rodrigues JFD, Zijp MC, Waaijers-van der Loop SL. The environmental impact of the Dutch health-care sector beyond climate change: an input-output analysis. Lancet Planet Health 2022; 6(12): e949–57.
10. Whitmee S, Haines A, Beyrer C, Boltz F, Capon AG, de Souza Dias BF, et al. Safeguarding human health in the Anthropocene epoch: report of The Rockefeller Foundation–Lancet Commission on planetary health. Lancet 2015; 386(10007): 1973–2028.
11. Mahamar A, Smit MJ, Sanogo K, Sinaba Y, Niambele SM, Sacko A, et al. Artemether–lumefantrine with or without single-dose primaquine and sulfadoxine–pyrimethamine plus amodiaquine with or without single-dose tafenoquine to reduce Plasmodium falciparum transmission: a phase 2, single-blind, randomised clinical trial in Ouelessebougou, Mali. Lancet Microbe 2024; 5(7): 633–44.
12. Legett R. Field Demonstration of High-Efficiency Ultra-Low-Temperature Laboratory Freezers. 2014.
13. Meerstein-Kessel L, Andolina C, Carrio E, Mahamar A, Sawa P, Diawara H, et al. A multiplex assay for the sensitive detection and quantification of male and female Plasmodium falciparum gametocytes. Malar J 2018; 17(1): 441.
14. Guinée JB, Gorrée M, Heijungs R, et al. Handbook on life cycle assessment. Operational guide to the ISO standards. Springer Science & Business Media; 2002: 692.
15. J D, C R. HealthcareLCA Database [Online Database] [cited 2024 May 07]. Available from: https://healthcarelca.com/

16. Ritchie H, Rosado P. Electricity Mix. Published online at OurWorldInData.org 2020 [01-08-2024]. Available from: https://ourworldindata.org/electricity-mix
17. Huijbregts MAJ, et al. ReCiPe 2016: A harmonized life cycle impact assessment method at midpoint and endpoint level. Report I: Characterization. National Institute for Public Health and the Environment; 2016.
18. Barret D. Estimating, monitoring and minimizing the travel footprint associated with the development of the Athena X-ray Integral Field Unit: An on-line travel footprint calculator released to the science community. Exp Astron (Dordr) 2020; 49(3): 183–216.
19. Laurent A, Weidema BP, Bare J, Liao X, de Souza DM, Pizzol M, et al. Methodological review and detailed guidance for the life cycle interpretation phase. J Ind Ecol 2020; 24(5): 986–1003.
20. Bousema J. Towards sustainable conferencing: comparing the carbon footprint of in-person, virtual and hybrid ASTM editions. ASTMH2021. 2021.
21. Sustainable Trials Study Group. Towards sustainable clinical trials. BMJ 2007; 334(7595): 671–3.
22. LaRoche JK, Alvarenga R, Collins M, Costelloe T, De Soete W, Faludi J, et al. Climate footprint of industry-sponsored clinical research: an analysis of a phase-1 randomised clinical study and discussion of opportunities to reduce its impact. BMJ Open 2024; 14(1): e077129.
23. Griffiths J, Fox L, Williamson PR, Low Carbon Clinical Trials Group. Quantifying the carbon footprint of clinical trials: guidance development and case studies. BMJ Open 2024; 14(1): e075755.
24. Mackillop N, Shah J, Collins M, Costelloe T, Öhman D. Carbon footprint of industry-sponsored late-stage clinical trials. BMJ Open 2023; 13(8): e072491.
25. Zero; DfESN. 2024 Government Gas Conversion Factors for company reporting. 2024.
26. Pencheon DC. Managing the environmental impact of research. Trials 2011; 12: 80.

Supplementary material

Please scan the QR code below to access the supplementary material for Chapter 3B:

CHAPTER 4

Artemether–lumefantrine–amodiaquine or artesunate–amodiaquine combined with single low-dose primaquine to reduce *Plasmodium falciparum* malaria transmission in Ouélessébougou, Mali: a five-arm, phase 2, single-blind, randomised controlled trial

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Summary

Background

Triple artemisinin-based combination therapies (TACTs) can delay the spread of antimalarial drug resistance. Artesunate–amodiaquine is widely used for uncomplicated *Plasmodium falciparum* malaria. We therefore aimed to determine the safety and efficacy of artemether–lumefantrine–amodiaquine and artesunate–amodiaquine with and without single low-dose primaquine for reducing gametocyte carriage and transmission to mosquitoes.

Methods

We did a five-arm, single-blind, phase 2 randomised controlled trial at the Ouélessébougou Clinical Research Unit of the Malaria Research and Training Centre of the University of Sciences, Techniques and Technologies of Bamako in Mali. Eligible participants were aged 10–50 years, with asymptomatic *P falciparum* microscopy-detected gametocyte carriage. Eligible participants were randomly allocated (1:1:1:1:1) to receive either artemether–lumefantrine, artemether–lumefantrine–amodiaquine, artemether–lumefantrine–amodiaquine plus primaquine, artesunate–amodiaquine, or artesunate–amodiaquine plus primaquine. Treatment regimens were administered on days 0, 1, and 2; primaquine was given as a single dose on day 0. All staff except the trial pharmacist and participants were masked to the treatment allocation. The primary outcome was the median percentage change in mosquito infection rate between pretreatment and 2 days after treatment initiation, assessed by direct membrane feeding assay. Data were analysed using a per-protocol analysis. This study is registered with ClinicalTrials.gov, NCT05550909.

Findings

Between Oct 16, 2022, and Dec 28, 2022, a total of 1249 individuals were screened; of whom, 100 were enrolled and randomly assigned to one of the five treatment groups (20 per group). Before treatment, 61 (61%) of 100 participants were infectious to mosquitoes, with a median of 7·3% (IQR 3·2 to 23·5) of mosquitoes becoming infected. Among infectious participants, the median percentage reduction in mosquito infection rate between pretreatment and 2 days after treatment was 100% (IQR 100 to 100) in the artemether–lumefantrine (p=0·0018), artemether–lumefantrine–amodiaquine (p=0·0018), and artemether–lumefantrine–amodiaquine plus primaquine (p=0·0009) treatment groups. In the artesunate–amodiaquine group the median percentage reduction in mosquito infection rate was only 32% (IQR −10·9 to 79·4; p=0·19),

whereas a 100% median reduction was seen in the artesunate–amodiaquine plus primaquine group (IQR 100 to 100; p=0·0009). At day 2, two (10%) of 20 participants in the artemether–lumefantrine group, two (11%) of 19 in the artemether–lumefantrine–amodiaquine group, and 15 (75%) of 20 in the artesunate–amodiaquine group infected any number of mosquitoes whereas no infected mosquitoes were observed at this timepoint in the groups with primaquine. 85 (85%) of 100 participants had a total of 262 adverse events during follow-up; of which, 181 (69%) were categorised as mild and 81 (31%) as moderate. No serious adverse events were reported.

Interpretation

Our findings support the effectiveness of artemether–lumefantrine alone or as part of TACT for preventing nearly all human–mosquito malaria parasite transmission within 48 h. By contrast, substantial transmission was observed following treatment with artesunate–amodiaquine. The addition of a single low dose of primaquine blocks transmission to mosquitoes rapidly regardless of schizonticide.

Research in context

Evidence before this study

We searched PubMed on Nov 13, 2023, with no publication date or language restrictions, for studies assessing the post-treatment transmission of artemether–lumefantrine–amodiaquine with the following search terms: "([Artemether-lumefantrine] OR [Coartem] OR [Riamet])" AND "([Amodiaquine] OR [Flavoquine] OR [Triple ACT] OR [TACT] OR [Triple Artemisinin-based Combination therapy] OR [Triple therapy])" AND "([Plasmodium falciparum])" AND "([Gametocytocidal] OR [Gametocytes])" AND "([Transmission])". The following search terms were added to search for studies using artemether–lumefantrine–amodiaquine plus primaquine: AND "([Primaquine] OR [Jasoprim] OR [Malirid] OR [Neo-Quipenyl] OR [Pimaquin] OR [Primachina] OR [Primacin] OR [Primaquina] OR [Remaquin])". A second search was done for studies assessing the post-treatment transmission of artesunate–amodiaquine, with the following search terms: "([Artesunate-amodiaquine] OR [Camoquin] OR [Coarsucam] OR [Artesunat Plus] OR [TesquinCare])" AND "([Plasmodium falciparum])" AND "([Gametocytocidal] OR [Gametocytes])" AND "([Transmission])". The aforementioned search terms for primaquine were added to search for studies using artesunate–amodiaquine plus primaquine.

The initial search (without the primaquine search terms) yielded nine studies: seven did not assess the combination of artemether–lumefantrine–amodiaquine and two were non-clinical trials. After addition of primaquine search terms, only one study was found, which assessed the effectiveness and post-treatment gametocyte density of four artemisinin-based combination therapies (ACTs) with or without primaquine; however, the combination of artemether–lumefantrine–amodiaquine was not tested. The second search found 26 studies: seven assessed safety and gametocyte carriage after artesunate–amodiaquine treatment but did not include mosquito feeding assays, 15 only tested artesunate and amodiaquine separately or in combination with other drugs, and four were non-clinical trials. The duration of gametocyte carriage, determined by microscopy, observed in these seven trials after treatment with artesunate–amodiaquine ranged from 21 days to persisting past 28 days after treatment initiation. Narrowing this search to include studies assessing artesunate–amodiaquine in combination with primaquine identified only two relevant trials, both of which assessed safety and efficacy against gametocytes determined by microscopy, but neither performed mosquito feeding assays. Both studies found that after artesunate–amodiaquine plus primaquine treatment gametocyte densities decreased to zero within 21–28 days.

Added value of this study

Our study provides valuable data for the extent of transmission after artemether–lumefantrine, artemether–lumefantrine–amodiaquine, and artesunate–amodiaquine in a highly infectious population sample. This study is the first assessment of artemether–lumefantrine–amodiaquine with and without single low-dose primaquine on gametocyte densities and transmission, using mosquito feeding assays. We show that artemether–lumefantrine–amodiaquine has potent transmission-blocking activity, even in the absence of primaquine. However, gametocyte densities declined more rapidly when primaquine was added and this combination blocks transmission even after gametocyte enrichment of the mosquito blood meal. We also provide the first evidence of continued transmission up until day 28 after artesunate–amodiaquine. Notably, we provide the first data demonstrating that the addition of a single-low dose of primaquine to artesunate–amodiaquine completely annuls transmission by day 2 after treatment. Lastly, we concentrated gametocytes in the mosquito blood meal to gain insights into the transmission-blocking mechanisms of antimalarial drugs.

Implications of all the available evidence

Our study findings are consistent with previous evidence that artemether–lumefantrine has potent transmission-blocking activity. We found that the addition of amodiaquine to artemether–lumefantrine did not influence gametocyte densities or transmission. However, the addition of a single-low dose of primaquine to the triple ACTs achieved a near-complete clearance of gametocytes by day 7, which is a more rapid clearance than previously observed after artemether–lumefantrine plus primaquine. In line with this finding, gametocyte enrichment enhanced transmission in the artemether–lumefantrine and artemether–lumefantrine–amodiaquine groups at day 2, but not in the group with added primaquine. This finding provides evidence that all gametocytes and transmission are completely annulled post-artemether–lumefantrine–amodiaquine plus primaquine treatment.

In addition, this study's findings contribute to the expanding body of research supporting the incorporation of a single low-dose primaquine regimen with ACT as an immediate measure to halt the further transmission of *Plasmodium falciparum* malaria. The available data for the effects of artesunate–amodiaquine alone provide a resource to policy makers considering treatment options, and our findings on the efficacy of artesunate–amodiaquine with a single-low does primaquine support WHO's recommendation of combining ACTs with single low-dose primaquine to prevent transmission in areas aiming to eliminate malaria or fighting the spread of antimalarial drug resistance.

Introduction

Malaria morbidity and mortality remains unacceptably high.[1] The emergence and spread of partial resistance against artemisinin derivatives, the main component of artemisinin-based combination therapies (ACTs), in southeast Asia[2,3] and east Africa[4,5] is threatening to increase malaria cases and deaths. Antimalarial treatments designed to slow the spread of resistance are therefore needed, either through novel combinations of existing drugs or supplementation with drugs that have specific effects on gametocytes, the sexual life stages responsible for maintaining parasite transmission. For optimal use, the understanding of how effective current and future antimalarials combat gametocytes is essential, and how this effect translates into reductions in transmission to mosquitoes.

Triple ACTs (TACTs) combine an existing ACT with a second partner drug that is slowly eliminated, to reduce the likelihood of incomplete parasite clearance and thus delay the spread of artemisinin resistance.[6] Artemether–lumefantrine–amodiaquine is a TACT that has been proven safe, well tolerated, and efficacious for the treatment of uncomplicated *Plasmodium falciparum* malaria, including in areas with artemisinin and partner drug resistance.[7,8] The effect of artemether–lumefantrine–amodiaquine on mature gametocytes and infectivity is unknown. Artesunate–amodiaquine is the first-line ACT for uncomplicated *P falciparum* malaria in many countries,[9] but its efficacy in reducing transmission has not been directly tested. Studies assessing gametocyte carriage after artesunate–amodiaquine reported persistent gametocyte carriage after treatment for 21 days or more, but without transmission assays the infectivity of these persisting gametocytes cannot be confirmed.[10–12]

Although artemisinin-based treatments have superior gametocytocidal properties to non-artemisinin-based treatments,[13] with artemether–lumefantrine being the most potent,[14] the transmission-reducing activities of ACTs vary widely.[14–16] By contrast, the 8-aminoquinoline primaquine is a potent gametocytocidal drug that, at a single low-dose (0·25 mg/kg), blocks transmission within 48 h of treatment. Since 2015, WHO recommends the addition of a single low-dose of primaquine to ACTs to reduce *P falciparum* transmission.[17] The gametocytocidal and transmission-reducing activities of single low-dose primaquine have been assessed in combination with dihydroartemisinin–piperaquine, pyronaridine–artesunate, and artemether–lumefantrine,[14–16,18,19]; however, combining artemether–lumefantrine–amodiaquine or artesunate–amodiaquine with a single low-dose primaquine for *P falciparum* transmission reduction has not yet been tested.

In this study, we aimed to determine the safety and efficacy of artemether–lumefantrine–amodiaquine and artesunate–amodiaquine with and without single low-dose primaquine for reducing the transmission of *P falciparum* gametocytes.

Methods

Study design and participants

We did a five-arm, single-blind, phase 2 randomised controlled trial at the Ouélessébougou Clinical Research Unit of the Malaria Research and Training

Centre (MRTC) of the University of Sciences, Techniques and Technologies of Bamako in Mali. Ouélessébougou is a commune that includes the town of Ouélessébougou and 44 surrounding villages, which have a total of approximately 50 000 inhabitants. Malaria transmission is highly seasonal, tied to the rainy season occurring from July to November. In children older than 5 years, the prevalence of *P falciparum* malaria varies between 50% and 60% and the prevalence of gametocytes varies between 20% and 25% during the transmission season. 2 days before the start of enrolment, the study team met with community leaders, village health workers, and heads of households from each village, before the commencement of screening, to explain the study and obtain verbal assent to undertake screening. Village health workers subsequently used a door-to-door approach to inform all available households of the date and location where consenting and screening would take place.

We included participants in the trial if they met the following criteria: positive for *P falciparum* gametocytes by microscopy (ie, ≥1 gametocytes observed in a thick film against 500 white blood cells, equating to 16 gametocytes per µL with a standard conversion of 8000 white blood cells per µL); absence of other non-*P falciparum* species on the blood film; haemoglobin density of 10 g/dL or more; aged between 10 and 50 years; bodyweight of 80 kg or less; no clinical signs of malaria, defined by fever (≥37·5°C); and no signs of acute, severe, or chronic disease. The exclusion criteria included pregnancy (tested at enrolment by urine test) or lactation, allergies to any of the study drugs, use of other medication (except for paracetamol or aspirin, or both), use of antimalarial drugs over the past week, history of prolongation of the corrected QT interval, documented or self-reported history of cardiac conduction problems or epileptic seizures, and blood transfusion in the last 90 days. A full list of the exclusion criteria is detailed in the appendix. We chose to recruit only asymptomatic individuals to increase the likelihood of observing high gametocyte densities.[20]

Before screening and study enrolment, participants provided written informed consent (≥18 years) or written parental consent (10–17 years). In addition to parental consent, oral assent was sought for individuals aged 10–17 years. Ethical approval was granted by the Ethics Committee of the University of Sciences, Techniques and Technologies of Bamako (Bamako, Mali; 2022/244/CE/USTTB), and the Research Ethics Committee of the London School of Hygiene & Tropical Medicine (London, UK; 28014). The study protocol is provided in the appendix.

Randomisation and masking

Participants were randomly allocated (1:1:1:1:1) to five treatment groups: artemether–lumefantrine; artemether–lumefantrine–amodiaquine; artemether–lumefantrine–amodiaquine plus primaquine; artesunate–amodiaquine; and artesunate–amodiaquine plus primaquine. An independent MRTC statistician randomly generated the treatment assignment using Stata (version 16), which was linked to participant identification numbers. The statistician prepared sealed, opaque envelopes with the participant identification number on the outside and treatment assignment inside, which were sent to the MRTC study pharmacist. Study participants were aware of the allocated treatment. The study pharmacist provided treatment and was consequently not masked to treatment assignment; staff involved in assessing safety, infectivity, and laboratory outcomes were masked.

Procedures

Artesunate–amodiaquine and artemether–lumefantrine treatment (Guilin Pharmaceutical, Shanghai, China) was administered over 3 days (days 0, 1, and 2) as per manufacturer instructions. Participants in the artemether–lumefantrine–amodiaquine groups were treated with standard doses (Guilin Pharmaceutical, Shanghai, China) over 3 days (days 0, 1, and 2) as per manufacturer instructions. A single dose of 0·25 mg/kg primaquine (ACE Pharmaceuticals, Zeewolde, Netherlands) was administered on day 0 in parallel with the first dose of ACT or TACT, as described previously.[19] Details about the dosing of these antimalarials are shown in supplementary information 1.

Participants received a full clinical and parasitological examination on days 2, 7, 14, 21, and 28 after receiving the first dose of the study drugs (supplementary figure 1). Giemsa-stained thick film microscopy was performed as described previously,[19] with gametocytes counted against 500 white blood cells and asexual stages counted against 200 white blood cells. Total nucleic acids were extracted for molecular gametocyte quantification using a MagNAPure LC automated extractor (Total Nucleic Acid Isolation Kit-High Performance; Roche Applied Science, Indianapolis, IN, USA). Female and male gametocytes were quantified in a multiplex reverse transcriptase quantitative PCR (RT-qPCR) assay (supplementary table 1).[21] Samples were classified as negative for a particular gametocyte sex if the RT-qPCR quantified density of gametocytes of that sex was less than 0·01 gametocytes per µL (ie, one gametocyte per 100 µL of blood sample). Haemoglobin density

(g/dL) was measured from fingerprick samples using a haemoglobin analyser (HemoCue; AB Leo Diagnostics, Helsingborg, Sweden) or using an automatic haematology analyser (HumaCount 5D; Human Diagnostics Worldwide, Wiesbaden, Germany) from venous blood samples. Additional venous blood samples were taken for biochemical and infectivity assessments on days 0, 2, 7, and 14 in all treatment groups (supplementary figure 1). Concentrations of aspartate aminotransferase, alanine aminotransferase, and blood creatinine were measured using the automatic biochemistry analyser Human 100 (Human Diagnostics Worldwide, Wiesbaden, Germany). For each assessment of infectivity, about 75 locally insectary-reared female *Anopheles gambiae (s.l.)* mosquitoes were allowed to feed for 15–20 min on venous blood samples (Lithium Heparin VACUETTE tube; Greiner Bio-One, Kremsmünster, Austria) through a prewarmed glass membrane feeder system (Coelen Glastechniek, Weldaad, Netherlands). Mosquitoes that had taken no bloodmeal or a partial bloodmeal were discarded; surviving blood-fed mosquitoes were dissected on the seventh day after feeding. Midguts were stained with 1% mercurochrome and examined for the presence and density of oocysts by one expert microscopist; positive midguts were confirmed by a second expert microscopist.

To investigate whether early post-treatment transmission-blocking was due either to insufficient gametocyte densities or drug-induced sterilisation effects, a separate venous blood sample (from baseline [day 0] and day 2 only) was processed by magnetic-activated cell sorting to enrich its gametocyte content before mosquito feeding in transmission assays. Gametocytes in the infected whole blood sample were concentrated by magnetic-activated cell sorting using a QuadroMACS separator and magnetic-activated cell sorting LS columns (MiltenyiBiotech, Bisley, UK) as previously described.[22] Briefly, MACS LS columns were equilibrated with 1 mL of warm incomplete medium, followed by 3 mL of infected whole blood and 2 mL medium wash. LS columns were then removed from the magnet, and gametocytes were eluted in 4 mL of warm medium. Flow-through and gametocyte fractions were then centrifuged (2000 rotations per min for 5 min at 37°C). The medium was carefully removed, and the gametocyte pellet was resuspended in 450 µL warm malaria naive serum and 600 µL of the same participants packed cells. The entire magnetic-activated cell sorting procedure was done in a 37°C cabinet incubator.

Outcomes

The primary outcome was median percentage change in mosquito infection rate between pretreatment and 2 days after treatment initiation. Secondary outcomes were mosquito infection metrics (infectious participants [ie, infected any number of mosquitoes], mosquito infection rate, and oocyst density) at prespecified timepoints (days 0, 2, 7, 14, 21, and 28); gametocyte and asexual parasite prevalence, density, gametocyte circulation time, area under the curve (AUC) of gametocyte density over time, and sex ratio (ie, proportion of gametocytes that were male or female); and safety assessments including incidence of clinical and laboratory adverse events. Differences in all transmission metrics, gametocyte, asexual stages, and safety outcomes were compared between matched treatment groups (ie, artemether–lumefantrine *vs* artemether–lumefantrine–amodiaquine and artemether–lumefantrine–amodiaquine plus primaquine, artesunate–amodiaquine *vs* artesunate–amodiaquine plus primaquine) as secondary outcomes.

Primary and secondary analyses of mosquito infection rate and oocyst density metrics were performed on participants infectious at baseline, but are shown for all participants in supplementary table 3. Exploratory outcomes included mosquito infection metrics after gametocyte enrichment, for within and between treatment group comparison. Gametocyte infectivity was assessed as an exploratory outcome using logistic regression models adjusted for gametocyte density, wherein the shape of the relationship between gametocyte density and mosquito infection rate was estimated using fractional polynomials.

Adverse events were graded by the study clinician for severity (mild, moderate, or severe) and relatedness to study medication (unrelated or unlikely, possibly, probably, or definitely related). A reduction in haemoglobin concentration of 40% or more from baseline was categorised as a haematological severe adverse event. An external data safety and monitoring committee was assembled before the trial. Safety data were discussed after enrolment of 50 participants, and after the final follow-up visit of the last participant.

Statistical analysis

Sample size was informed by previous trials in the study setting using a mixed effects logistic regression model that accounted for correlation between mosquito observations from the same participant[14–16,18,19] and expecting a reduction in infectivity of 90% as previously detected for a single low-dose

of primaquine.[15,19] When including 20 participants per group and dissecting 50 mosquitoes per participant per timepoint, we calculated 92% empirical power to detect more than 85% reduction in infectivity with a one-tailed test with an α of 0·05 level of significance. The sample size was not designed for between-group comparisons and any comparison of transmission-blocking effects between groups is secondary and limited to matched treatment groups. Mosquito infectivity was assessed at three levels: the percentage of participants infectious to any number of mosquitoes (ie, infectious participants), the proportion of mosquitoes infected with any number of oocysts (ie, mosquito infection rate), and the mean number of oocysts in a sample of mosquitoes (ie, oocyst density).

The proportion of infectious participants and the prevalence of gametocytes and asexual stage parasites were compared between treatment groups using one-sided Fisher's exact tests and within groups using McNemar tests. Mosquito infection rate was compared within groups (relative to baseline) by Wilcoxon sign rank test (*Z* score) and between groups by linear regression adjusted for baseline mosquito infection rate (*t* score, coefficient with 95% CI). For all direct membrane feeding assays (before and after gametocyte enrichment), the proportion of infectious participants was compared between groups (direct membrane feeds before gametocyte concentration as reference) and within groups (relative to baseline) using one-sided Fisher's exact tests. Haemoglobin concentrations were compared using paired *t* tests (*t* score) for within-group analyses and linear regression adjusted for baseline levels of each measure for between-group analyses (*t* score, coefficient with 95% CI). Percentage change from baseline was analysed using two-sample *t* tests for between-group analysis and paired *t* tests (*t* score) for within-group analysis.

The proportion of gametocytes that were male was analysed for all values with total gametocyte densities of 0·2 gametocytes per µL or more, ensuring accurate quantification of sex ratios. Gametocyte circulation time was calculated to determine the mean number of days that a mature gametocyte circulates in the blood before clearance, using a deterministic compartmental model that assumes a constant rate of clearance and has a random effect to account for repeated measures on participants, as described previously.[23] Differences in circulation time between groups and between gametocyte sexes were estimated in the model. Statistical theory shows that these parameter estimates follow a t-distribution. AUC of gametocyte density per participant over time was calculated using the linear trapezoid method and was analysed

by fitting linear regression models to the $\log_{10}$ adjusted AUC values, with adjustment for baseline gametocyte density (*t* score, coefficient with 95% CI). All other analyses of quantitative data were done using Wilcoxon sign rank tests (*Z* score) and Wilcoxon rank-sum tests (*Z* score). All comparisons were defined before study completion and analyses were not adjusted for multiple comparisons. For all analyses, the threshold for statistical significance was set at p<0·05.

Statistical analysis was conducted using Stata (version 17.0) and SAS (version 9.4). Data visualisation was performed using the R-based *ggplot2* package (version 4.3.2) and Stata-based graphics (version 17.0). This trial is registered with ClinicalTrials.gov, number NCT05550909.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Oct 16, 2022, and Dec 28, 2022, a total of 1249 eligible individuals were screened; of whom, 100 were enrolled and randomly assigned to one of the five treatment groups (20 participants per group; figure 1). Participant characteristics were similar between the treatment groups, although the proportion of infectious participants at baseline was higher in the artesunate–amodiaquine group (tables 1, 2). The primary outcome was recorded on day 2 of follow-up, with 98 (98%) of 100 participants completing this study visit (one in the artemether–lumefantrine–amodiaquine group and one in the artemether–lumefantrine–amodiaquine plus primaquine group did not complete this visit). 96 (96%) of 100 participants completed all visits to day 28 (two in the artemether–lumefantrine–amodiaquine, one in the artemether–lumefantrine–amodiaquine plus primaquine, and one in the artesunate–amodiaquine group did not complete all visits).

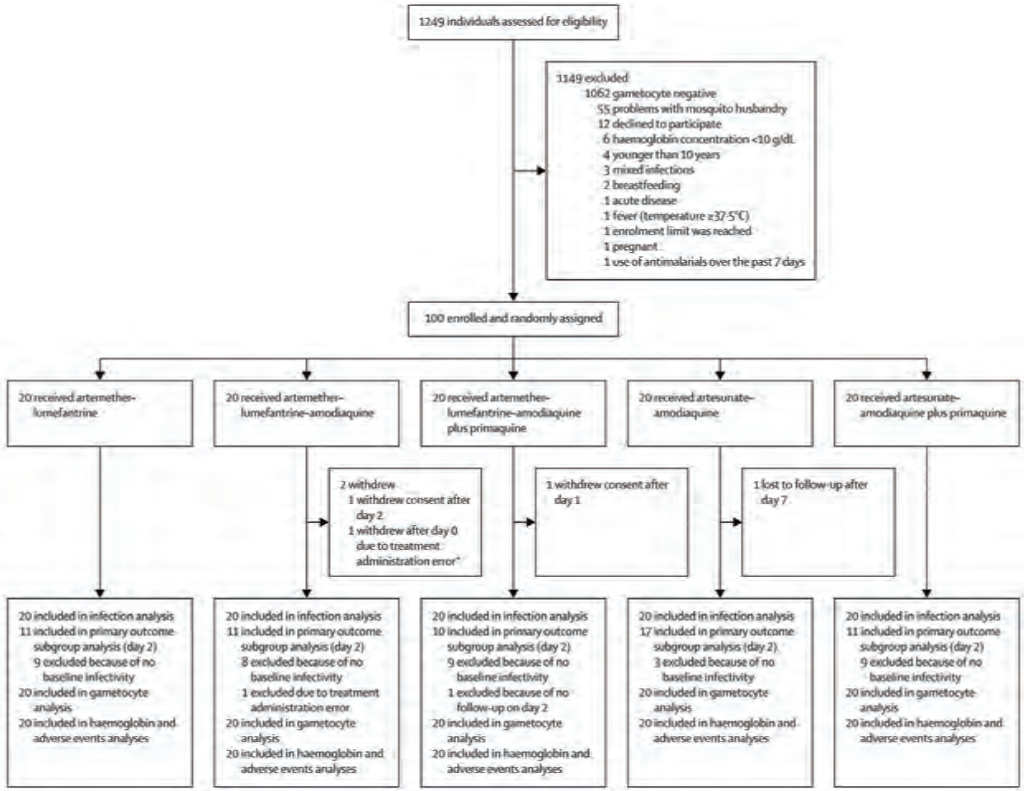

Figure 1. Trial profile. 96 (96%) of 100 participants completed all visits to day 28 (two [10%] of 20 in the artemether–lumefantrine–amodiaquine group, one [5%] in the artemether–lumefantrine–amodiaquine plus primaquine group, and one [5%] in the artesunate–amodiaquine plus primaquine group did not complete all visits). *One participant randomly allocated to the artemether–lumefantrine–amodiaquine group was given erroneous treatment on day 1. All measures following this error were removed from analysis.

	Artemether–lumefantrine (n=20)	Artemether–lumefantrine–amodiaquine (n=20)	Artemether–lumefantrine–amodiaquine plus primaquine (n=20)	Artesunate–amodiaquine (n=20)	Artesunate–amodiaquine plus primaquine (n=20)
Age (years)	13·0 (11·0–18·5)	13·0 (11·5–28·0)	13·0 (11·0–15·0)	12·0 (10·0–16·0)	12·5 (11·5–20·0)
Sex					
Female	11 (55%)	10 (50%)	12 (60%)	11 (55%)	7 (35%)
Male	9 (45%)	10 (50%)	8 (40%)	9 (45%)	13 (65%)
Haemoglobin (g/dL)	11·8 (11·5–13·8)	12·1 (11·3–12·6)	11·8 (11·1–12·1)	11·4 (10·9–12·3)	11·7 (11·2–12·8)
Gametocyte prevalence	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)
Gametocyte density (parasites per µL)	31·0 (19·9–92·7)	28·6 (11·5–130·5)	42·3 (11·8–97·0)	52·5 (33·6–129·0)	24·8 (10·7–115·2)
Asexual parasite prevalence	10 (50%)	6 (30%)	5 (25%)	8 (40%)	8 (40%)
Asexual parasite density (parasites per µL)	37·9 (0·0–300·0)	0·0 (0·0–79·8)	0·0 (0·0–37·6)	0·0 (0·0–1654·9)	0·0 (0·0–720·0)

Table 1. Baseline characteristics before treatment. Data are median (IQR) or n (%).

The median number of mosquitoes dissected in an individual mosquito feeding experiment was 60 (IQR 54 to 64). Before treatment, 61 (61%) of 100 participants were infectious to mosquitoes (17 participants randomly allocated to the artesunate–amodiaquine group and 11 in all other treatment groups), with a median of 7·3% (IQR 3·2 to 23·5) of mosquitoes becoming infected. The median number of oocysts per infected mosquito was 1·3 (IQR 1·0 to 2·7). At day 2 a significant within-person reduction in mosquito infection rate relative to baseline was observed in all groups except for the artesunate–amodiaquine group, with a median percentage reduction in mosquito infection rate of 100% (IQR 100 to 100) in the artemether–lumefantrine (p=0·0018), artemether–lumefantrine–amodiaquine (p=0·0018), and artemether–lumefantrine–amodiaquine plus primaquine (p=0·0009) treatment groups. In the artesunate–amodiaquine group the median percentage reduction in mosquito infection rate was only 32% (IQR −10·9 to 79.4; p=0·19), whereas a 100% median reduction was seen in the artesunate–amodiaquine plus primaquine group (IQR 100 to 100; p=0·0009). At day 2, two (10%) of 20 participants in the artemether–lumefantrine group, two (11%) of 19 in the artemether–lumefantrine–amodiaquine group, and 15 (75%) of 20 in the artesunate–amodiaquine group infected any number of mosquitoes. No participants remained infectious to mosquitoes at day 2 in the treatment groups with primaquine (table 2). At all timepoints after day 2, infectious participants were only found in the artesunate–amodiaquine group; seven (35%) of 20 at day 7, three (16%) of 19 at day 14, and one (5%) of 19 at days 21 and 28 (figure 2; supplementary table 2). Mosquito infection data for all participants, regardless of baseline infectivity, are presented in the supplementary table 3.

	Infectious participants	Median mosquito infection rate	Median percentage reduction in mosquito infection rate	p value*	p value†
Baseline					
Artemether–lumefantrine	11/20 (55%)	4·5% (3·3–44·1)	..	Ref	..
Artemether–lumefantrine–amodiaquine	11/20 (55%)	10·9% (3·3–32·3)	..	Ref	..
Artemether–lumefantrine–amodiaquine plus primaquine	11/20 (55%)	4·1% (2·1–8·8)	..	Ref	..
Artesunate–amodiaquine	17/20 (85%)	7·3% (1·9–23·5)	..	Ref	..
Artesunate–amodiaquine plus primaquine	11/20 (55%)	9·3% (1·8–36·2)	..	Ref	..
Day 2					
Artemether–lumefantrine	2/20 (10%)	0% (0–0)	100% (100 to 100)	0·0018	Ref
Artemether–lumefantrine–amodiaquine	2/19 (11%)	0% (0–0)	100% (100 to 100)	0·0018	1·0000
Artemether–lumefantrine–amodiaquine plus primaquine	0/19	0% (0–0)	100% (100 to 100)	0·0009	0·15
Artesunate–amodiaquine	15/20 (75%)	5% (1·5–9·7)	31·7% (−10·87 to 79·39)	0·19	Ref
Artesunate–amodiaquine plus primaquine	0/20	0% (0–0)	100% (100 to 100)	0·0009	0·0001

Table 2. Median percentage reduction in mosquito infection rate for participants infectious before treatment. Data are n/N (%) or median (IQR), unless otherwise specified. Participants were classed as infectious if direct membrane feeding assays resulted in at least one mosquito with any number of oocysts. All values are for participants who were infectious to mosquitoes before treatment. The range of median percentage reduction in mosquito infection rate between pretreatment and 2 days after treatment was 83·1 to 100 in the artemether-lumefantrine group, 82·4 to 100 in the artemether–lumefantrine–amodiaquine group, 100 to 100 in the artemether-lumefantrine-amodiaquine plus primaquine group, −112·6 to 100 in the artesunate–amodiaquine group, and 100 to 100 in the artesunate-amodiaquine plus primaquine group.

* Within-group comparison of median reduction in mosquito infection rate by Wilcoxon signed rank test (day 0 as reference, primary outcome).

† Between-group comparison of median reduction in mosquito infection rate (ie, artemether-lumefantrine *vs* artemether-lumefantrine–amodiaquine and artemether–lumefantrine–amodiaquine plus primaquine, artesunate-amodiaquine vs artesunate–amodiaquine plus primaquine) by Wilcoxon rank-sum test. Full details about mosquito feeding assay outcomes are summarised in supplementary table 2. Ref=reference.

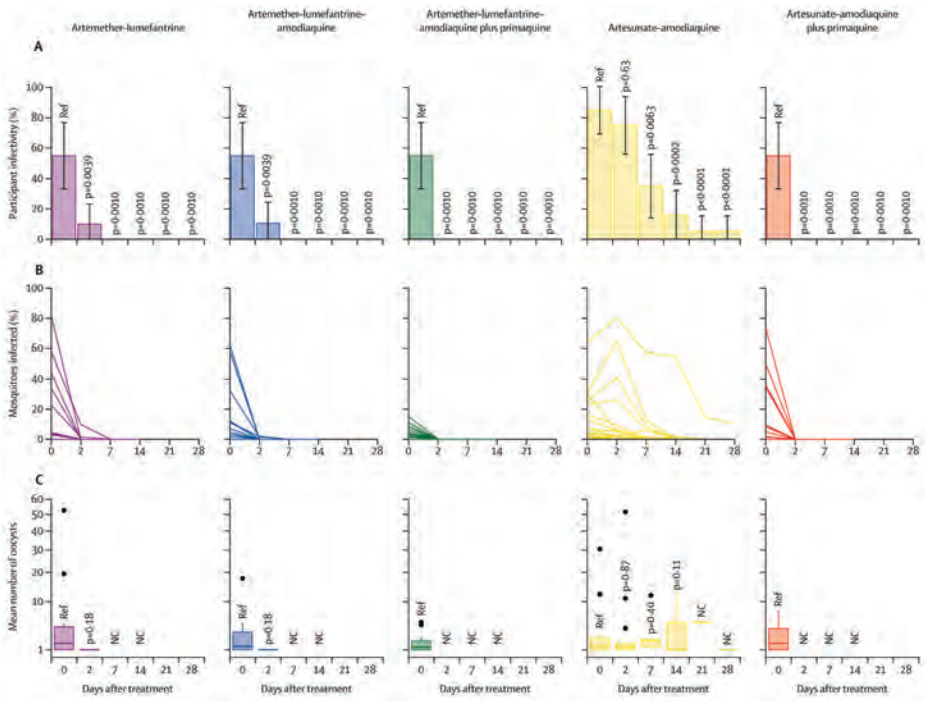

Figure 2. Participant infectivity and proportion of mosquitoes infected in direct membrane feeding assays. (A) The proportion of infectious individuals was compared within treatment groups using McNemar tests. The denominator for participant infectivity is the total number of participants still enrolled at the given timepoint, rather than the number tested for infectivity at that timepoint. Infectivity assays were discontinued after 14 days when a participant did not infect any mosquitoes at two subsequent timepoints and were thereafter considered non-infectious. Mosquito feeding assays at days 21 and 28 were only done in the artesunate–amodiaquine group (seven [37%] of 19 at day 21 and three [16%] of 19 at day 28). Error bars are 95% CIs. (B) Each line represents one participant. Statistical analyses are summarised in supplementary table 2. (C) Box plots show the median (central line), IQR (box limits), upper and lower quartiles plus 1·5 IQR (whiskers), and outliers for mean oocyst densities in infected mosquitoes within each participant. The mean number of oocysts was compared to baseline within treatment groups using the Wilcoxon sign-rank test. NC=not calculable. Ref=reference.

Gametocyte enrichment by magnetic-activated cell sorting was performed on 95 blood samples collected before treatment on day 0 (baseline) and 94 blood samples that were collected on the second day after treatment initiation. Overall, gametocyte enrichment increased mosquito infection rates by a mean of 7·3% (SD 16·3; supplementary figure 2). Comparing direct membrane feeds before enrichment with those after, the percentage of infectious participants increased in the enrichment-boosted group in all treatment groups at baseline (supplementary figure 2); whereas, at day 2, the percentage of infectious participants increased for all treatment groups except for artemether–

lumefantrine–amodiaquine plus primaquine, in which all participants remained non-infectious. In the artesunate–amodiaquine plus primaquine group, two initially non-infectious participants transmitted to one and four mosquitoes following gametocyte enrichment.

Asexual parasite densities, measured by microscopy, decreased rapidly after treatment initiation, with only one (5%) of 20 participants in both the artemether–lumefantrine and artesunate–amodiaquine groups retaining asexual stages at day 2; whereas, in all other treatment groups, no asexual parasites were observed after treatment initiation (supplementary table 5). Gametocyte densities declined over time in all treatment groups, although much more rapidly in those who received primaquine, with median gametocyte densities of 13·3 gametocytes per µL (IQR 5·6–21·0), 6·9 (1·1–49·9), and 31·7 (7·3–61·6) at day 7 in the groups without primaquine compared with median densities of 0·0 (0·0–0·0) and 0·2 (0·0–0·9) in the groups with primaquine (supplementary figure 3 and supplementary table 6). 18 (95%) of 19 participants treated with artesunate–amodiaquine were still gametocyte positive (>1 gametocyte per 100 µL) at the final day of follow-up (day 28), whereas 16 (80%) of 20 in the artemether–lumefantrine and 13 (72%) of 18 in the artemether–lumefantrine–amodiaquine groups remained gametocyte positive at the same timepoint. Only one (5%) of 19 participants in the artemether–lumefantrine–amodiaquine plus primaquine group and one (5%) of 20 in the artesunate–amodiaquine plus primaquine group had persisting gametocytes at day 28. Total gametocyte circulation time was estimated at 6·1 days (95% CI 5·4–6·9) in the artemether–lumefantrine group, 6·0 days (5·2–6·8) in the artemether–lumefantrine–amodiaquine group, and 2·6 (2·1–3·1) in the artemether–lumefantrine–amodiaquine plus primaquine group (supplementary table 7); the same measure was estimated at 7·9 days (95% CI 6·7–9·3) in the artesunate–amodiaquine group and 3·3 days (2·8–3·8) in the artesunate–amodiaquine plus primaquine group. Gametocyte sex ratios showed a male bias from day 2 after starting treatment in the artemether–lumefantrine, artemether–lumefantrine–amodiaquine, and artemether–lumefantrine–amodiaquine plus primaquine groups; and from day 7 in the artesunate–amodiaquine plus primaquine group, with significantly more males in this group than in the artesunate–amodiaquine alone group (median proportion of male gametocytes of 1·0 [IQR 0·9–1·0] *vs* 0·6 [0·4–0·7]; p=0·0002; supplementary 6 and 8, supplementary figure 4). Too few gametocytes persisted to make conclusions about absolute per-gametocyte infectivity (supplementary table 9).

	Total (n=100)	Artemether-lumefantrine (n=20)	Artemether-lumefantrine-amodiaquine (n=20)	Artemether-lumefantrine-amodiaquine plus primaquine (n=20)	Artesunate-amodiaquine (n=20)	Artesunate-amodiaquine plus primaquine (n=20)
All adverse events	85 (85%)	18 (90%)	15 (75%)	18 (90%)	16 (80%)	18 (90%)
p value	0·612*	Ref	0·407†	1·000†	Ref	0·661†
Mild related adverse events	47 (47%)	12 (60%)	7 (35%)	8 (40%)	7 (35%)	13 (65%)
p value	0·178*	Ref	0·205†	0·343†	Ref	0·113†
Moderate related adverse events	26 (26%)	2 (10%)	7 (35%)	7 (35%)	8 (40%)	2 (10%)
p value	0·055*	Ref	0·065127†	0·127†	Ref	0·065†
Severe related adverse events	0	0	0	0	0	0
p value	NC	Ref	NC	NC	ref	NC

Table 3. Frequency of adverse events. Data are n (%), unless otherwise specified. If there were multiple episodes of adverse events per participant, the highest grade and most likely related to treatment is summarised in this table.

* p values are from Fisher's exact tests for differences in proportion of participants with an adverse event between all groups.

† p values are from Fisher's exact tests for differences in proportion of participants with an adverse event between the artemether-lumefantrine-amodiaquine or artemether-lumefantrine-amodiaquine plus primaquine groups and the artemether-lumefantrine reference group and artesunate-amodiaquine plus primaquine group and the artesunate-amodiaquine reference group. Ref=reference group. NC=not calculable.

A significant difference was observed in the within-group reduction in mean haemoglobin density in all treatment groups at day 2 compared with baseline; however, by day 7, the haemoglobin concentrations had normalised in all groups and were comparable to baseline (supplementary table 10 and supplementary figure 5). The greatest reduction in mean haemoglobin density in any treatment group or timepoint was 5·6% (SD 2·0; 95% CI 3·6–7·5) in the artesunate–amodiaquine group at day 2. No significant decreases were observed in percentage change in haemoglobin compared with baseline between treatment groups at any timepoint. The greatest reduction in haemoglobin density in any participant was 25·2% (from 14·3 g/dL at baseline to 10·7 g/dL at day 21 in a participant in the artemether–lumefantrine group). The lowest observed haemoglobin density in any participant and timepoint was 9 g/dL at baseline in a participant in the artesunate–amodiaquine group. No severe laboratory abnormalities occurred; all possibly drug-related laboratory abnormalities normalised on the subsequent visit (supplementary table 11).

Overall, 85 (85%) of 100 participants had a total of 262 adverse events during follow-up; of which, 181 (69%) were categorised as mild and 81 (31%) as moderate (table 3; supplementary table 12). No severe adverse events or serious adverse events occurred during the trial. The most common treatment-related adverse event was mild or moderate headache, which occurred in 43 (43%) of 100 participants (six [14%] in the artemether–lumefantrine group, nine [21%] in the artemether–lumefantrine plus amodiaquine group, 11 [26%] in the artemether–lumefantrine–amodiaquine plus primaquine group, eight [19%] in the artesunate–amodiaquine group, and nine [21%] in the artesunate–amodiaquine plus primaquine group). No significant differences were observed between treatment groups in the proportion of participants who experienced any adverse event (p=0·61), mild treatment-related adverse events (p=0·18), or moderate treatment-related adverse events (p=0·055) at any study visit.

Discussion

To our knowledge, this study is the first clinical trial designed to test the gametocytocidal and transmission-blocking properties of the ACT artemether–lumefantrine–amodiaquine with and without primaquine and of artesunate–amodiaquine with and without primaquine. Within 48 h of treatment, transmission was greatly reduced in the artemether–lumefantrine and artemether–

lumefantrine–amodiaquine groups, and completely annulled in both treatment groups with primaquine. By contrast, transmission to mosquitoes continued in a minority of participants until day 28 after treatment with artesunate–amodiaquine alone.

Calls for malaria eradication and the emergence and spread of drug resistance have reinforced the need to assess the effects of antimalarial drugs on gametocytes and their infectiousness.[17,24] The addition of a second partner drug to ACTs could substantially delay the emergence and spread of artemisinin resistance and treatment failure. Lumefantrine and amodiaquine provide mutual protection against resistance development, and deployment of the TACT artemether–lumefantrine–amodiaquine is expected to extend the viability of artemisinin derivatives and both partner drugs.[6] This study was not designed to investigate the clinical efficacy of TACT, nor had the study site recorded any partial artemisinin resistance at the time of the study. We found that both treatments with artemether–lumefantrine alone and with amodiaquine greatly reduced transmission by day 2 after treatment. The addition of a single low-dose primaquine only marginally enhanced this transmission-blocking effect. Gametocyte densities minimally differed between artemether–lumefantrine and artemether–lumefantrine–amodiaquine, but we observed a near complete clearance of gametocytes by day 7 in the group with an added single low-dose of primaquine. These observations align with recent data indicating that artemether–lumefantrine has potent transmission-blocking effects.[14]

Artesunate–amodiaquine is the first-line treatment for uncomplicated *P falciparum* malaria in many countries, yet its effect on mature gametocytes and transmission is unclear. In line with previous studies,[10,11] we found that gametocyte carriage persisted in all participants treated with artesunate–amodiaquine until the end of follow-up (day 28) and three (16%) of 19 participants (three [18%] of 17 participants infectious at baseline) were still infectious to mosquitoes 14 days after initiation of treatment, with one participant remaining infectious until the end of follow-up (day 28). Moreover, one (5%) of 20 participants was infectious on day 2 but not at baseline, and one (5%) participant was infectious on day 7, but not at baseline or day 2. As transmission to mosquitoes involves an inherent stochastic element, the observation of no infected mosquitoes at one timepoint cannot rule out low levels of infectivity. This pattern suggests a possible role for differences in the drug susceptibility or exposure at different gametocyte developmental stages—ie, immature gametocytes might be released from sequestration in the bone marrow or spleen after treatment. The addition of a single low-dose of

primaquine resulted in an enhanced clearance of gametocytes and achieved in a near-total reduction of transmission potential within 48 h.

The exploratory assay of magnetic gametocyte enrichment showed that the percentage of infectious participants increased at day 2 after treatment start in all groups except for the artemether–lumefantrine–amodiaquine plus primaquine group, although this increase was non-significant. This finding suggests that in all ACT only groups, the initial lack of infectivity is due to low gametocyte densities or sex ratio distortion rather than the sterilisation of either gametocyte sex. In the primaquine groups, the enrichment results were contradictory: the addition of primaquine to artemether–lumefantrine–amodiaquine blocked all transmission at day 2 even after gametocyte enrichment, suggestive of primaquine sterilising gametocytes before reducing their numbers significantly.[25] Conversely, in the artesunate–amodiaquine plus primaquine group, two participants who were not infectious in standard feeding assays became infectious after gametocyte concentration. Although these samples are small and other factors such as variations in primaquine concentration have not been measured, this finding might indicate varying primaquine efficacy with different artemisinin therapies. Of relevance is that in the process of gametocyte enrichment, human plasma is replaced by malaria-naive serum, thereby removing potentially transmission-modulating antibodies or drugs that might affect parasite development upon mosquito ingestion.

The emergence of transmission after treatment, which was observed in the artesunate–amodiaquine group in standard feeding assays, has been seen previously after artemisinin[14] and non-artemisinin treatments.[14,26] Taken together with the observation that two initially non-infectious participants became infectious after gametocyte enrichment in the artesunate–amodiaquine plus primaquine group on day 2, we hypothesise that artesunate–amodiaquine might have lower efficacy on or exposure to immature, developing gametocytes than artemether–lumefantrine, and that gametocytes released from sequestration after treatment would be unaffected by primaquine's active metabolites, which only circulate for a few hours (median elimination half-life of 4·7 hours).[27] In addition, although artemether–lumefantrine–amodiaquine without primaquine prevents nearly all mosquito infections within 48 h, gametocytes in *PfKelch13* mutant infections might preferentially survive artemisinin exposure and infect mosquitoes.[28] Our data support the suggestion from WHO's malaria policy and advisory group to expand the focus on reducing

parasite transmission with a single low-dose of primaquine in areas where partial artemisinin resistance has been detected.[29]

Previous studies reported a higher frequency of side-effects with the combination of partner drugs lumefantrine and amodiaquine than with lumefantrine alone,[7,8] including vomiting, nausea, vertigo, and mild bradycardia. We did not see an increase in vomiting or nausea, and only a slight increase in vertigo related to the drug treatment, from one adverse event in the artemether–lumefantrine group, to three and six adverse events in the artemether–lumefantrine–amodiaquine groups with and without primaquine, respectively. Overall, all drug regimens were well tolerated, and no instances of cardiac adverse events or severe side-effects were reported.

Our study had some limitations. For instance, we assessed many secondary outcomes, and their interpretations therefore require caution because of issues of multiple testing. In addition, we recruited participants who had high densities of gametocytes, consistent with previous studies with similar outcomes and at the same study site.[15,16,18,19] This approach allowed us to collect robust data for post-treatment transmissibility but does not represent the average gametocyte-infected participant. Consequently, our estimates of persistence of transmissible gametocytes primarily demonstrate the effect of antimalarial drugs on the transmission potential stemming from a comparatively small subset of highly infectious participants; although these effects would be the most important group for the drug regimens to work in. Lastly, the public health significance of our study findings needs to be validated through community trials focused on transmission outcomes. Mass administrations of primaquine or other gametocytocidal compounds (eg, alongside seasonal malaria chemoprophylaxis) might be necessary to achieve reductions in transmission at the community level.[30] Conversely, given the negligible cost of primaquine, absence of safety concerns, and no obvious alternative, a compelling argument exists to add primaquine to slow the transmission of drug-resistant parasites.

In conclusion, our findings show that artemether–lumefantrine–amodiaquine can prevent nearly all mosquito infections, but reveal considerable post-treatment transmission after artesunate–amodiaquine. The addition of a single low-dose of primaquine is a safe and effective addition to artemether–lumefantrine–amodiaquine and artesunate–amodiaquine for blocking *P falciparum* transmission. Enriching the gametocyte content of mosquito blood meals in transmission assays

shows that viable male and female gametocytes can persist at densities too low to result in mosquito infection at physiological concentrations, after treatment in the artemether–lumefantrine, artemether–lumefantrine–amodiaquine, artesunate–amodiaquine and artesunate–amodiaquine plus primaquine groups, but not in the artemether–lumefantrine–amodiaquine plus primaquine group. This finding strengthens the argument for the addition of a single-low dose of primaquine to block the transmission of artemisinin resistant gametocytes.

Contributors

LNV, WS, AM, MJS, ADo, CD, and ADi conceived the study and developed the study protocol. AM, WS, LNV, MJS, KS, YS, SMN, OMD, RSD, MD, SOM, AY, AS, SK, SS, ADe, YD, SFT, CD, and ADi implemented the trial. KT did the molecular analyses. LNV, AM, and WS verified the raw data. LNV and WS analysed the data. LNV, AM, WS, MJS, CD, and ADi wrote the first draft of the manuscript. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication. All authors read and approved the final manuscript.

Data sharing

Anonymised data reported in the manuscript will be made available to investigators who provide a methodologically sound proposal to the corresponding author. The study protocol is available in the appendix. The data from this trial are accessible on Clinical Epidemiology Database Resources.

Declaration of interests

We declare no competing interests.

Acknowledgments

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References

1. WHO. World malaria report 2023. World Health Organization, Geneva, 2023. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022 (accessed December 13, 2023).
2. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in Plasmodium falciparum Malaria. N Engl J Med 2009; 361: 455–67.
3. van der Pluijm RW, Imwong M, Chau NH, et al. Determinants of dihydroartemisinin–piperaquine treatment failure in Plasmodium falciparum malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. Lancet Infect Dis 2019; 19: 952–61.
4. Uwimana A, Legrand E, Stokes BH, et al. Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. Nat Med 2020; 26: 1602–8.
5. Asua V, Conrad MD, Aydemir O, et al. Changing prevalence of potential mediators of aminoquinoline, antifolate, and artemisinin resistance across Uganda. J Infect Dis 2020; 223: 985–94.
6. Nguyen TD, Gao B, Amaratunga C, et al. Preventing antimalarial drug resistance with triple artemisinin-based combination therapies. Nat Commun 2023; 14: 4568.
7. van der Pluijm RW, Tripura R, Hoglund RM, et al. Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated Plasmodium falciparum malaria: a multicentre, open-label, randomised clinical trial. Lancet 2020; 395: 1345–60.
8. Peto TJ, Tripura R, Callery JJ, et al. Triple therapy with artemether–lumefantrine plus amodiaquine versus artemether–lumefantrine alone for artemisinin-resistant, uncomplicated falciparum malaria: an open-label, randomised, multicentre trial. Lancet Infect Dis 2022; 22: 867–78.
9. WHO. World malaria report 2021. World Health Organization, Geneva, 2021. https://apps.who.int/iris/handle/10665/350147 (accessed April 4, 2022).
10. WWARN Gametocyte Study Group. Gametocyte carriage in uncomplicated Plasmodium falciparum malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data. BMC Med 2016; 14: 79.
11. Adjuik M, Agnamey P, Babiker A, et al. Amodiaquine–artesunate versus amodiaquine for uncomplicated Plasmodium falciparum malaria in African children: a randomised, multicentre trial. Lancet 2002; 359: 1365–72.
12. Rakotoarisoa MA, Fenomanana J, Dodoson BT, et al. Comparative effect of artemether–lumefantrine and artesunate–amodiaquine on gametocyte clearance in children with uncomplicated Plasmodium falciparum malaria in Madagascar. Malar J 2022; 21: 331.
13. Lelièvre J, Almela MJ, Lozano S, et al. Activity of clinically relevant antimalarial drugs on Plasmodium falciparum mature gametocytes in an ATP bioluminescence "transmission blocking" assay. PLoS One 2012; 7: e35019.
14. Mahamar A, Smit MJ, Sanogo K, et al. Artemether–lumefantrine with or without single-dose primaquine and sulfadoxine–pyrimethamine plus amodiaquine with or without single-dose tafenoquine to reduce Plasmodium falciparum transmission: a phase 2, single-blind, randomised clinical trial in Ouélessébougou, Mali. Lancet Microbe 2024; 5: 633–44.

15. Stone W, Mahamar A, Sanogo K, et al. Pyronaridine–artesunate or dihydroartemisinin–piperaquine combined with single low-dose primaquine to prevent Plasmodium falciparum malaria transmission in Ouélessébougou, Mali: a four-arm, single-blind, phase 2–3, randomised trial. Lancet Microbe 2022; 3: e41–51.
16. Stone W, Mahamar A, Smit MJ, et al. Single low-dose tafenoquine combined with dihydroartemisinin–piperaquine to reduce Plasmodium falciparum transmission in Ouélessébougou, Mali: a phase 2, single-blind, randomised clinical trial. Lancet Microbe 2022; 3: e336–47.
17. WHO. Global malaria programme: WHO policy brief on single-dose primaquine as gametocytocide in Plasmodium falciparum malaria. World Health Organization, Geneva, 2014. https://www.who.int/publications/i/item/WHO-HTM-GMP-2015.1 (accessed November 13, 2023).
18. Dicko A, Brown JM, Diawara H, et al. Primaquine to reduce transmission of Plasmodium falciparum malaria in Mali: a single-blind, dose-ranging, adaptive randomised phase 2 trial. Lancet Infect Dis 2016; 16: 674–84.
19. Dicko A, Roh ME, Diawara H, et al. Efficacy and safety of primaquine and methylene blue for prevention of Plasmodium falciparum transmission in Mali: a phase 2, single-blind, randomised controlled trial. Lancet Infect Dis 2018; 18: 627–39.
20. Andolina C, Rek JC, Briggs J, et al. Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. Lancet Infect Dis 2021; 21: 1568–78.
21. Meerstein-Kessel L, Andolina C, Carrio E, et al. A multiplex assay for the sensitive detection and quantification of male and female Plasmodium falciparum gametocytes. Malar J 2018; 17: 441.
22. Reuling IJ, Stone WJR, van de Vegte-Bolmer M, et al. Concentration of Plasmodium falciparum gametocytes in whole blood samples by magnetic cell sorting enhances parasite infection rates in mosquito feeding assays. Malar J 2017; 16: 315.
23. Bousema T, Okell L, Shekalaghe S, et al. Revisiting the circulation time of Plasmodium falciparum gametocytes: molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. Malar J 2010; 9: 136.
24. Feachem RGA, Chen I, Akbari O, et al. Malaria eradication within a generation: ambitious, achievable, and necessary. Lancet 2019; 394: 1056–112.
25. Stone W, Sawa P, Lanke K, et al. A molecular assay to quantify male and female Plasmodium falciparum gametocytes: results from 2 randomized controlled trials using primaquine for gametocyte clearance. J Infect Dis 2017; 216: 457–67.
26. Enosse S, Butcher GA, Margos G, et al. The mosquito transmission of malaria: the effects of atovaquone–proguanil (Malarone) and chloroquine. Trans R Soc Trop Med Hyg 2000; 94: 77–82.
27. Mukaka M, Onyamboko MA, Olupot-Olupot P, et al. Pharmacokinetics of single low dose primaquine in Ugandan and Congolese children with falciparum malaria. EBioMedicine 2023; 96: 104805.
28. Witmer K, Dahalan FA, Delves MJ, et al. Transmission of artemisinin-resistant malaria parasites to mosquitoes under antimalarial drug pressure. Antimicrob Agents Chemother 2020; 65: e00898–20.
29. WHO. WHO Malaria Policy Advisory Group (MPAG) meeting report. World Health Organization, Geneva, 2023. https://iris.who.int/bitstream/handle/10665/368391/9789240074385-eng.pdf?sequence=1 (accessed November 15, 2023).
30. Gerardin J, Eckhoff P, Wenger EA. Mass campaigns with antimalarial drugs: a modelling comparison of artemether–lumefantrine and DHA–piperaquine with and without primaquine as tools for malaria control and elimination. BMC Infect Dis 2015; 15: 144.

Supplementary Material

Please scan the QR code below to access the supplementary material for Chapter 4:

Part 2: Active and passive immunisation to reduce *Plasmodium falciparum* transmission

CHAPTER 5

A Pfs48/45-based vaccine to block *Plasmodium falciparum* transmission: phase 1, open-label, clinical trial

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Abstract

Background

The stalling global progress in malaria control highlights the need for novel tools for malaria elimination, including transmission-blocking vaccines. Transmission-blocking vaccines aim to induce human antibodies that block parasite development in the mosquito and mosquitoes becoming infectious. The Pfs48/45 protein is a leading *Plasmodium falciparum* transmission-blocking vaccine candidate. The R0.6C fusion protein, consisting of Pfs48/45 domain 3 (6C) and the N-terminal region of *P. falciparum* glutamate-rich protein (R0), has previously been produced in *Lactococcus lactis* and elicited functional antibodies in rodents. Here, we assess the safety and transmission-reducing efficacy of R0.6C adsorbed to aluminium hydroxide with and without Matrix-M™ adjuvant in humans.

Methods

In this first-in-human, open-label clinical trial, malaria-naïve adults, aged 18–55 years, were recruited at the Radboudumc in Nijmegen, the Netherlands. Participants received four intramuscular vaccinations on days 0, 28, 56 and 168 with either 30 µg or 100 µg of R0.6C and were randomised for the allocation of one of the two different adjuvant combinations: aluminium hydroxide alone, or aluminium hydroxide combined with Matrix-M1™ adjuvant. Adverse events were recorded from inclusion until 84 days after the fourth vaccination. Anti-R0.6C and anti-6C IgG titres were measured by enzyme-linked immunosorbent assay. Transmission-reducing activity of participants' serum and purified vaccine-specific immunoglobulin G was assessed by standard membrane feeding assays using laboratory-reared *Anopheles stephensi* mosquitoes and cultured *P. falciparum* gametocytes.

Results

Thirty-one participants completed four vaccinations and were included in the analysis. Administration of all doses was safe and well-tolerated, with one related grade 3 adverse event (transient fever) and no serious adverse events occurring. Anti-R0.6C and anti-6C IgG titres were similar between the 30 and 100 µg R0.6C arms, but higher in Matrix-M1™ arms. Neat participant sera did not induce significant transmission-reducing activity in mosquito feeding experiments, but concentrated vaccine-specific IgGs purified from sera collected two weeks after the fourth vaccination achieved up to 99% transmission-reducing activity.

Conclusions
R0.6C/aluminium hydroxide with or without Matrix-M1™ is safe, immunogenic and induces functional Pfs48/45-specific transmission-blocking antibodies, albeit at insufficient serum concentrations to result in transmission reduction by neat serum. Future work should focus on identifying alternative vaccine formulations or regimens that enhance functional antibody responses.

Trial registration
The trial is registered with ClinicalTrials.gov under identifier NCT04862416.

Background

With almost 250 million infections and approximately 600 thousand deaths per year, malaria remains a global health priority.[1,2] The renewed focus on malaria elimination has increased the priority of research into interventions to block malaria transmission.[1,2] By interrupting the highly efficient transmission of malaria parasites by mosquito vectors from infected to susceptible individuals, a significant reduction in the number of secondary infections can be achieved, resulting in an overall reduction in disease and mortality.[3] Malaria transmission-blocking vaccines (TBVs) aim to interrupt transmission to, or the development of parasites in, the mosquito vector by vaccination of the human host.4 Deployment of TBVs is considered to be an efficient element in an integrated program of anti-malarial interventions, aiming to reduce the overall malaria burden, contain drug resistance, and move towards malaria elimination.[2,5]

Transmission of malaria is dependent on the uptake of male and female gametocytes, the sexual reproductive forms of the Plasmodium parasite, in the mosquito blood meal and their subsequent fertilisation in the mosquito midgut. Transmission-blocking vaccine candidate antigens are expressed during gamete stages (Pfs48/45 and Pfs230), zygote and ookinete stages (Pfs25 and Pfs28), or alternatively by the mosquito midgut (AnAPN1).[6] Antibodies against these antigens can interfere with parasite development in the mosquito when taken up in a bloodmeal, preventing onward transmission. Until now, only Pfs25 and Pfs230 have reached clinical evaluation of which the latter has recently shown promising, durable functional activity in Malian adults.[7,8] The sexual stage Pfs48/45 antigen has a critical role in parasite fertilisation and is a lead candidate for a *P. falciparum* TBV as naturally-acquired human antibodies

that target this protein can exert potent transmission-reducing activity (TRA).[9-11] Pfs48/45 is expressed by gametocytes, but while these still reside within the human host, this protein remains hidden from the immune system inside the host red blood cell, and therefore cannot be targeted by antibodies. When a mosquito takes a gametocyte-containing bloodmeal, however, the parasite emerges from the red blood cell and Pfs48/45 becomes accessible to antibodies present in the blood meal. Antibodies targeting the C-terminal domain of P48/45 (D3 or 6C) can prevent oocyst and ultimately sporozoite development.[10,12,13] Recent findings show that administration of TB31F, a monoclonal antibody targeting the 6C region of Pfs48/45, to malaria naïve trial participants resulted in high-level TRA of their sera in standard membrane feeding assays (SMFA).[14] In such assays, cultured *P. falciparum* gametocytes are fed to laboratory-reared *Anopheles* mosquitoes in the presence or absence of test sera or antibodies. TRA is expressed as the reduction of oocyst count in mosquitoes fed on gametocytes in the presence of the test serum compared to a non-serum control.

The R0.6C fusion protein consists of the C-terminal 6-cysteine domain of Pfs48/45 (6C or D3) and the N-terminal region of asexual stage *P. falciparum* glutamate-rich protein GLURP (R0) produced in Lactococcus lactis.[15] Preclinical immunisation studies with adjuvants approved for use in humans revealed that R0.6C, when formulated with either aluminium hydroxide or Matrix-M1™ alone, induced modest TRA in SMFA. The addition of Matrix-M1™ to R0.6C adsorbed on aluminium hydroxide substantially increased immunogenicity relative to R0.6C administration with either adjuvant alone, resulting in strong TRA.[16] Here, we report the safety, tolerability, immunogenicity and TRA of R0.6C/aluminium hydroxide without or with Matrix-M1™, the first Pfs48/45-based *Plasmodium falciparum* TBV to be assessed in humans.

Methods

Study design and population

This first-in-human, open-label, randomised trial was conducted at the Radboud University Medical Center (Nijmegen, the Netherlands). The study population comprised healthy, male and female malaria-naïve adults aged 18–55 years. All participants provided written informed consent prior to screening. Screening procedures included medical history, physical examination, urine toxicology screening, a pregnancy test for participants

of childbearing potential and blood collection for routine clinical laboratory testing of biochemical and haematological parameters, as well as HIV, hepatitis B, and hepatitis C serological screening. The trial protocol (research file number NL7666.000.21) received ethics and regulatory approval by the Netherlands' Central Committee on Research Involving Human Subjects (CCMO) and a positive marginal review for research with a medicinal product by the national competent authority (Ministry of Health, Welfare and Sport). The trial was registered at ClinicalTrials.gov, identifier NCT04862416 and EudraCT, identifier 2021–000017-17.

Study product

The R0.6C fusion protein is a chimaera consisting of the 6-cysteine C-terminal fragment of Pfs48/45 (D3, 6C) and the N-terminal region of asexual stage glutamate-rich protein GLURP (R0) produced in *Lactococcus lactis*.[15,17,18] The recombinant R0.6C protein is formulated in 10 mM HEPES, 2.5% glucose, 0.5 mM EDTA, 155 mM NaCl and absorbed to 8 µg aluminium hydroxide (Alhydrogel®, AlOH) per µg of R0.6C and stored at 2–8 °C. The study product was manufactured under current Good Manufacturing Practices[16] by Statens Serum Institut (Denmark) and vialed at Baccinex (Switzerland). Matrix-M1™ (hereafter referred to simply as Matrix-M) is a saponin-based adjuvant manufactured by Novavax AB, Sweden.[19,20]

Study procedures

Thirty-two participants were enrolled to receive four vaccinations intramuscularly in the deltoid muscle on alternating sides on days 0, 28, 56 and 168. Participants of childbearing potential were instructed to use adequate contraception throughout the study period. Participants were divided over the four study arms that received either 30 µg or 100 µg of R0.6C, each either adjuvanted with AlOH alone or combined with Matrix-M; $n = 8$ per R0.6C dose and adjuvant combination (Fig. 1). In order to maintain the same ratio of AlOH to Matrix-M adjuvants, 30-µg R0.6C/AlOH doses were admixed with 15-µg Matrix-M and 100-µg R0.6C/AlOH doses were admixed with 49-µg Matrix-M. The volume of the administered study products ranged from 0.15 mL to 0.63 mL depending on dose and adjuvant. A sentinel enrolment group of 3 participants in each study arm started vaccinations. A minimum of 7 days after the first vaccination in the sentinel groups of the two 30-µg R0.6C study arms, an interim assessment for any dose-related safety concerns was conducted by an independent safety monitoring committee, before the remaining 5 participants in each 30-µg R0.6C study arm started vaccinations (consolidation enrolment

group) and before escalation to the 100-µg R0.6C dose. The same procedure with a sentinel enrolment group of 3 participants in each 100-µg R0.6C study arm, followed by a consolidation enrolment group of 5 participants in each study arm, was done for the 100 µg vaccinations. Adverse events (AEs) were collected at each study visit from the first vaccination until 84 days after the fourth vaccination. Blood samples for mosquito feeding assays and antibody measurements were taken at predefined timepoints throughout the study.

Randomisation

Participants were randomly allocated 1:1 to receive AlOH alone or AlOH + Matrix-M using a Mersenne-Twister random number generator implemented in R. Randomisation was stratified on R0.6C dose and sentinel/consolidation group. Participants and study personnel were all aware of allocation (dose/adjuvant combination).

Safety assessment

Per protocol, AEs were graded as mild/grade 1 (easily tolerated), moderate/grade 2 (interfering with daily activity), or severe/grade 3 (preventing daily activity), and in the case of fever as grade 1 (38.0–38.4 °C), grade 2 (38.5–38.9 °C) or grade 3 (≥ 39 °C). Additionally, local AEs are reported here according to the Food and Drug Administration (FDA) AE grading scale.[21] AEs were categorized by the International Classification of Diseases 10 code. Until 7 days after each vaccination, the following local adverse events were solicited: pain, pruritus, swelling, induration, and erythema at the injection site. Until 14 days after each vaccination, the following systemic adverse events were solicited: fever, headache, myalgia, fatigue, chills, and rash. Any other adverse events were categorized as unsolicited adverse events. For each adverse event, causality to the study procedures was categorized as not related, unlikely related, possibly related, probably related, or definitely related; where a dichotomous classification was required, the first two categories were together considered as unrelated and the latter three as related. Safety blood tests including haematology and biochemistry evaluations were performed at each study visit, except at the study visits 56 days after the third and fourth vaccinations.

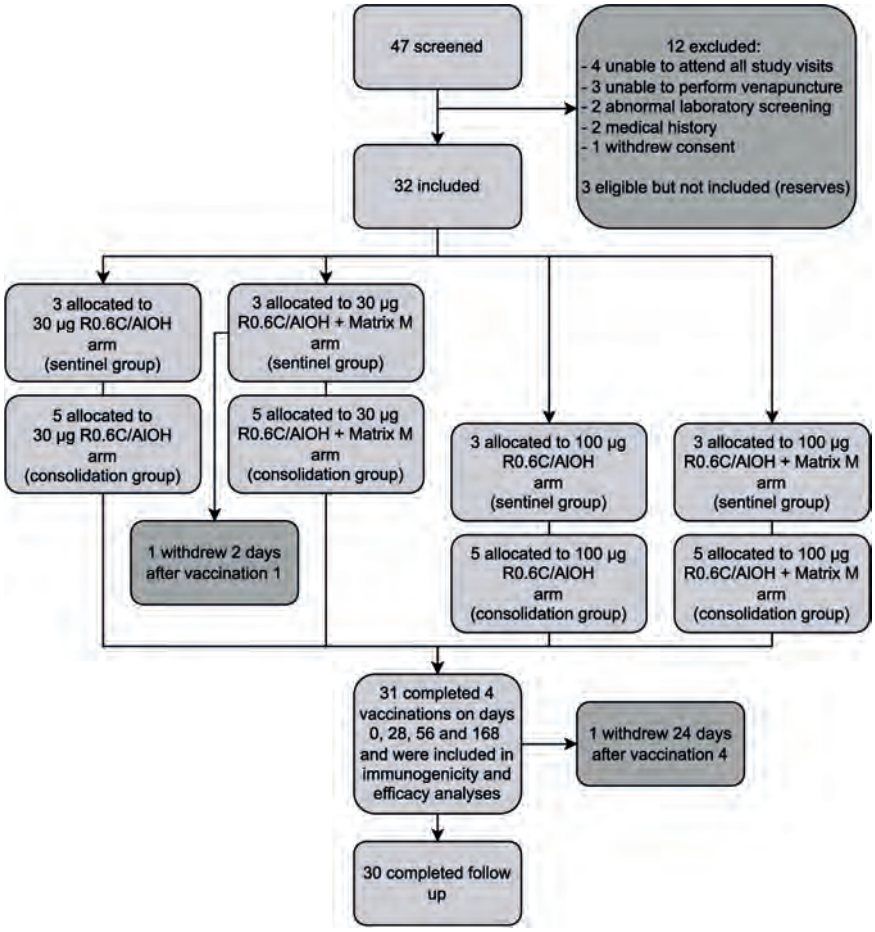

Figure 1. Screening and enrolment.

Quantification of vaccine-specific IgG concentrations

Serum concentrations of IgG antibodies against R0.6C and 6C antigens were quantified by enzyme-linked immunosorbent assays (ELISAs).[16,22] Nunc MaxiSorp™ 96-well plates (ThermoFisher) were coated overnight at 4 °C with 100 µl of 0.5 µg/mL antigen per well. Plates were blocked with 5% skimmed milk in PBS and subsequently incubated with diluted participant serum. Detection was done with 1:40,000 dilution goat anti-human IgG HRP (Invitrogen, Cat. No. 31412). Plates were developed by adding 100 µL tetramethylbenzidine and stopped with 50-µL 0.2M H2SO4. Absorbances were read at 450 nm on an iMark™ microplate absorbance reader (Bio-Rad). Analyses were performed using Auditable Data Analysis and Management System for ELISA (ADAMSEL FPL v1.1). Serially diluted TB31F monoclonal antibody with known

concentration served as a standard curve. The standard curve was plotted on a logarithmic scale and fitted to a power trend line (R2 > 0.99) and optical density measurements for each test sample (average of duplicates that were no more than 25% different) were converted to concentrations in µg/mL relative to the standard curve. For each seroconverted participant, the ratio of 6C/R0.6C antibody concentrations at each study timepoint was also calculated.

Purification of anti-R0.6C antibodies

Total IgG was purified using a 1 mL HiTrap(R) Protein G HP column (GE Healthcare) according to the manufacturer's instructions with few modifications: 6 mL of total citrate plasma was diluted with 6-mL binding buffer (PBS) and precipitated with 12 mL 2X ammonium sulfate (100%) for 30 min at room temperature. The samples were centrifugated at 3200 × g for 15 min at room temperature and the pellet was resuspended in 24 mL of ammonium persulfate (50% saturated). A second centrifugation was performed at 16,100 × g for 10 min and the pellet was finally dissolved in 24 mL PBS. The samples were filtered using a 0.45-µm filter before loading on to the HiTrap® Protein G HP column. After loading the samples, the column was washed with 15 mL of binding buffer. Total IgG was eluted with 1 mL fractions IgG elution Buffer (Thermo scientific[23]) in tubes containing 150-µL 1.0M Tris pH 8.8. Fractions containing IgG were pooled and buffer exchanged to 6 mL PBS using Vivaspin(R) 20 (30kDa MWCO) concentrators (Sartorius VS2022, Stonehouse, UK).

Anti-R0.6C antibodies were purified using n 1-mL HiTrap N-hydroxysuccinimide activated HP column (GE Healthcare) to which R0.6C was covalently coupled following the manufacturer's instructions.[16] Total IgG, purified from participant serum, was loaded on the column followed by a wash step with 15 mL of PBS. Bound anti-R0.6C antibodies were eluted with 6 mL IgG elution Buffer (Thermo scientific[23]) in tubes containing 150-µL 1.0M Tris pH 8.8. Fractions containing anti-R0.6C antibodies were pooled and buffer exchanged to 200 µL of 25% PBS using Vivaspin 20 (30kDa MWCO) concentrators. The final volume of samples containing R0.6C-specific antibodies thus equal 1/30th of the original plasma volumes.

Mosquito feeding experiments

SMFAs were used to determine the TRA of participants' sera and of affinity-purified anti-R0.6C antibodies, as described previously.[24] In short, 90 µL of participant's serum or 90 µL R0.6C antibodies added to 90 µL freeze dried fetal

calf serum (FCS), was mixed with 150 µL packed red blood cells and cultured *P. falciparum* NF54 gametocytes, and 30 µL naïve human serum containing active complement, before feeding to *Anopheles stephensi* (Sind-Kasur Nijmegen strain) mosquitoes. After 6–8 days, oocysts were counted in 20 fully-fed mosquitoes per feeding condition. TRA for participant sera was calculated as the reduction in oocysts compared to the participant's pre-immunisation control serum. Affinity-purified R0.6C-specific IgGs were tested in two independent SMFA experiments using FCS as a negative control.

Statistical analysis

Analyses were performed using SPSS V25 (IBM) and Graphpad Prism(R) V9.0.0. For comparison between study groups, the Mann–Whitney *U* test was used. Paired comparisons were performed with the Wilcoxon signed ranks test. Two-sided *p*-values < 0.05 were considered significant; Bonferroni correction for multiple testing was used where appropriate and as indicated. Individual level TRA of affinity purified R0.6C-specific IgGs was estimated using a mixed effects negative binomial regression model available as online data analysis tool.[25]

Results

Recruitment and study population

Thirty-two malaria-naïve adults were enrolled sequentially to the study arms receiving 30 µg R0.6C per dose ($n = 16$ total) and 100 µg R0.6C per dose ($n = 16$ total). Stratified randomisation (i.e. *within* each sentinel and consolidation group) resulted in balanced baseline characteristics between participants immunised with R0.6C/AlOH only or with R0.6C/AlOH + Matrix-M (Table 1) for both the low and high doses. Overall, 66% of the participants was female, the mean age of participants was 28 years (range 18–53) and their mean BMI was 23.2 kg/m^2 (range 18.7–29.3). One participant in the 30-µg R0.6C/AlOH + Matrix-M arm withdrew from the trial 2 days after their first vaccination, on the grounds of a hematoma resulting from venipuncture for routine safety blood collection; this AE was classified as mild and related to study procedures in general, but not to the investigational product itself. This participant is included in the safety analyses, but not in the immunological or functional analyses as no blood samples were collected for these endpoints. A second participant withdrew from the trial 24 days after receiving their fourth

vaccination due to personal circumstances unrelated to the trial. All 30 other participants received all four vaccinations and completed follow-up (Fig. 1).

Table 1. Baseline characteristics

	30 µg R0.6C/AlOH	30 µg R0.6C/AlOH + Matrix-M	100 µg R0.6C/AlOH	100 µg R0.6C/AlOH + Matrix-M
Participants (*n*)	8	8	8	8
Female/male (*n*)	5/3	4/3	6/2	6/2
Age (years)	23 (18–51)	25 (19–53)	22 (20–47)	22 (18–49)
Weight (kg)	73.5 (57.0–90.0)	73.7 (61.0–75.6)	68.4 (57.8–89.0)	65.0 (57.0–72.8)
BMI (kg/m^2)	24.2 (20.8–28.8)	22.2 (18.7–27.4)	23.1 (19.5–29.3)	22.6 (19.2–24.6)

Safety and tolerability

No serious adverse events occurred. Solicited adverse events were mostly local and mostly mild or moderate (Fig. 2; supplementary table 1 and supplementary figure 1 and 2). Fourteen out of 31 participants experienced some level of local reactogenicity after vaccinations #2, #3 and/or #4, consisting of erythema and induration or swelling (up to 22 cm in diameter), pruritus and/or pain. With the exception of one participant in the R0.6C/AlOH arm with mild symptoms, this local reactogenicity occurred only in participants who received vaccinations with R0.6C/AlOH + Matrix-M. Per protocol, these adverse events were graded mild or moderate based on disruption of daily activities, and resolved spontaneously within 3–4 days after onset. According to the more conservative current FDA AE severity grading scale,[21] five of these individual occurrences would be classified as grade 3, based on the diameter of erythema, induration and/or swelling. Notably, although local reactogenicity was observed only following the second or later vaccinations, there was no indication of increased reactogenicity upon subsequent vaccinations in a given participant. Only one systemic grade 3 adverse event occurred: a participant-reported 39.4 °C fever the second night after the fourth vaccination in the 100 µg R0.6C/AlOH + Matrix-M arm, which was considered probably related to vaccination and had resolved spontaneously by the next morning. There were nine laboratory abnormalities considered clinically significant, mostly eosinophilia, that accompanied the local reactions and resolved spontaneously (supplementary table 2).

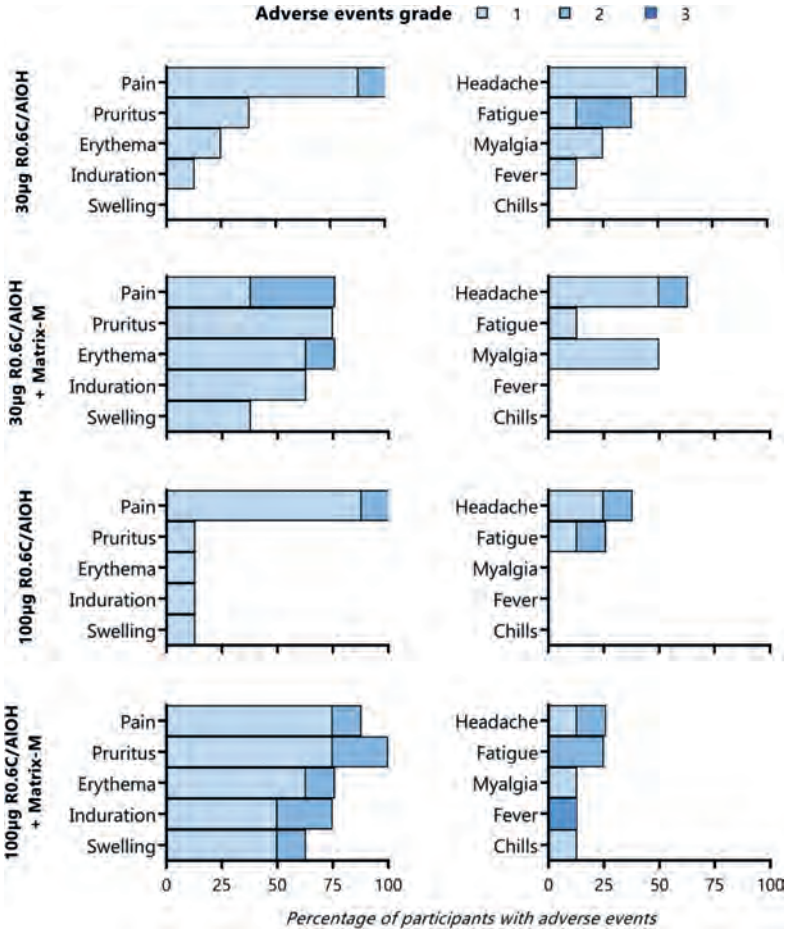

Figure 2. Solicited local and systemic adverse events. Per protocol, adverse events were graded as mild/grade 1 (easily tolerated), moderate/grade 2 (interfering with daily activity), or severe/grade 3 (preventing daily activity), and in the case of fever as grade 1 (38.0–38.4 °C), grade 2 (38.5–38.9 °C) or grade 3 (≥ 39 °C). Post hoc, local adverse events were also graded according to the Food and Drug Administration (FDA) Adverse Event grading scale (supplementary figure 2), to more conservatively reflect observed local reactogenicity. If there was more than one episode per participant, the highest grade adverse event was listed. *One participant withdrew from follow-up after the first immunisation and adverse events for this participant were recorded only until 2 days after the first immunisation. The solicited systemic adverse event 'rash' was not reported during the study.

Immunogenicity

Antibody responses against R0.6C and 6C recombinant proteins were detectable in all vaccinated participants, with the exception of one participant who received vaccinations with 30 µg R0.6C/AlOH (Fig. 3A, B; supplementary figure 3A). Anti-6C IgG concentrations were significantly (approximately

ten-fold) higher in the R0.6C/AlOH + Matrix-M study arms compared to the R0.6C/AlOH study arms on days I2 + 14 ($p < 0.001$), I3 + 14 ($p < 0.001$), I3 + 56 ($p = 0.001$), I4 + 14 ($p = 0.002$) and I4 + 56 ($p = 0.006$) after Bonferroni-correction for multiple comparisons (p-values of < 0.00625 were considered statistically significant), but not on days I1 + 14, I4 – 1 and I4 + 84. Anti-R0.6C IgG concentrations were significantly higher in the R0.6C/AlOH + Matrix-M study arms compared to the R0.6C/AlOH study arms on days I1 + 14, I3 + 14, I3 + 56, I4 – 1, I4 + 14, I4 + 56 and I4 + 84 ($p < 0.001$ for all timepoints) but not on I2 + 14. Within adjuvant groups, there was no significant difference in anti-6C or anti-R0.6C IgG concentrations between the 30 µg and 100 µg study arms at any timepoint after correction for multiple comparisons. Geometric mean IgG concentrations against both 6C and R0.6C induced by immunisation with R0.6C/AlOH alone increased somewhat after each subsequent immunisation. In contrast, IgG responses induced by R0.6C/AlOH + Matrix-M reached peak concentrations after only two immunisations and did not reach significantly higher concentrations after either the third or fourth immunisation (repeated measures ANOVA followed by pairwise comparison with Bonferroni correction). IgG responses against 6C as a fraction of total IgG responses against R0.6C remained stable over time in each dose/adjuvant arm from 14 days after the second immunisation onward. The geometric mean fraction was 10% in sera from participants immunised with R0.6C/AlOH + Matrix-M and significantly higher (geometric mean 40%; $p < 0.001$) in participants immunised with R0.6C/AlOH alone (Fig. 3C). Comparison of the slopes of regression lines from log transformed R0.6C and 6C IgG concentrations after the third and fourth vaccination revealed no significant differences in antibody decay rate ($p = 0.86$ and $p = 0.89$ respectively; supplementary figure 4).

Functional transmission-reducing activity

No statistically significant reduction in oocyst density was seen in SMFA experiments using sera collected at 14 days after either the third or fourth immunisation from participants in any of the four study arms, as compared to their own baseline sera (Fig. 4A). Since anti-6C IgG responses were detected in post-immunisation sera by ELISA, albeit at relatively low concentrations, additional SMFAs were performed post hoc with concentrated IgG from samples collected 14 days after the fourth immunisation from 6 participants with the highest such responses ($n = 2$ who received 100 µg R0.6C/AlOH with Matrix-M, $n = 2$ who received 100 µg R0.6C/AlOH alone and $n = 2$ who received 30 µg R0.6C/AlOH with Matrix-M). R0.6C-specific antibodies were purified on R0.6C-coated columns and concentrated to approximately 30 times the original

volume. These samples were tested in two independent SMFA experiments and had estimated TRAs of 22 till 99% (Fig. 4B). 6C IgG serum titres were associated with TRA (Spearman's ρ= 1.0, p = 0.0028, Fig. 4C).

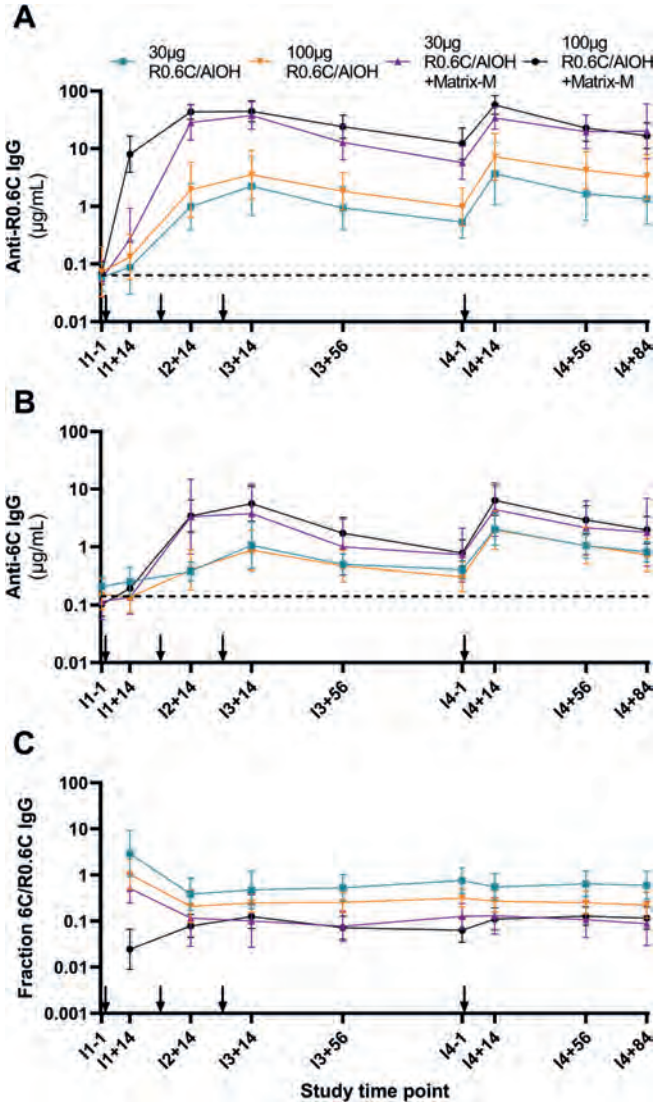

Figure 3. Anti-6C and anti-R0.6C IgG antibody responses over time. A) Geometric mean anti-R0.6C antibody concentrations per study arm. B) Geometric mean anti-6C antibody concentrations per study arm. C) Geometric mean IgG responses against 6C as a fraction of total IgG responses against R0.6C per study arm. IgG concentrations in A and B are calculated using serially diluted anti-6C antibody TB31F of known concentration as a reference. Arrows represent vaccinations. Error bars indicate 95% CI. Dashed lines indicate the geometric mean of pre-immunisation (baseline) serum samples.

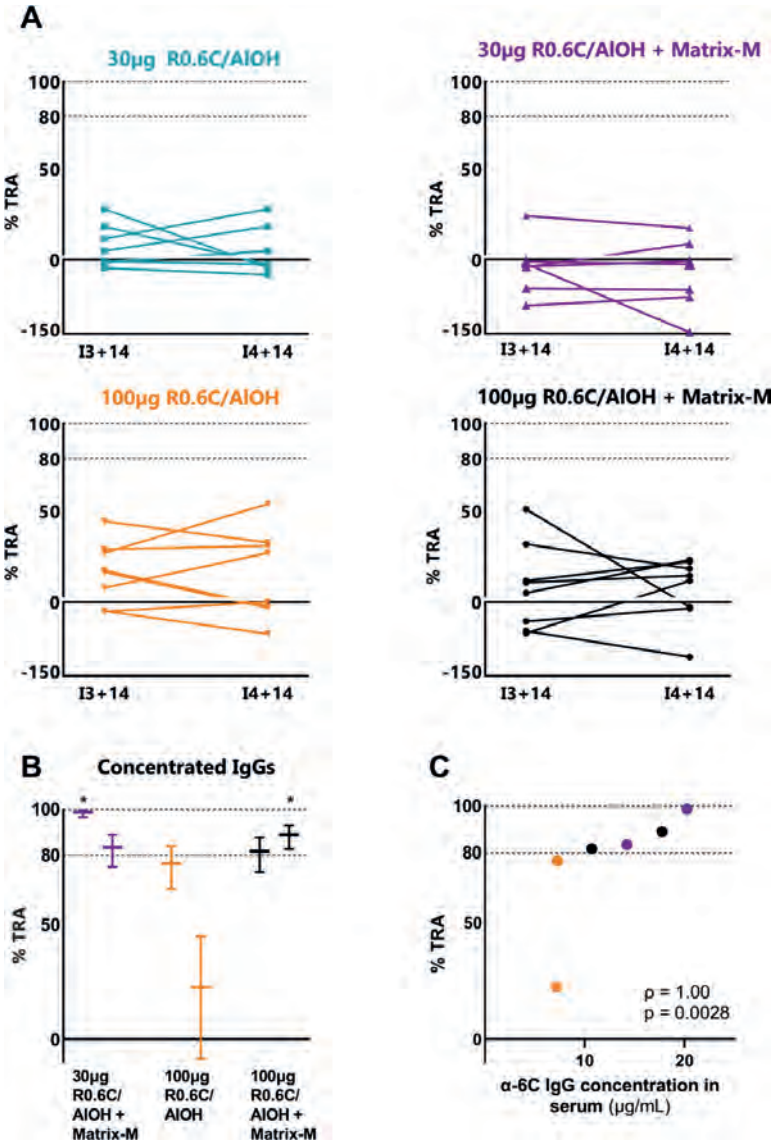

Figure 4. Functional transmission-reducing activity in SMFA. A) SMFA with participants' sera collected two weeks after the third and fourth immunisation. Each data point represents the transmission-reducing activity of participants' sera compared to their pre-immunisation sera. B) SMFA with purified and concentrated anti-R0.6C IgGs from sera of a selection of six participants collected at 2 weeks after the fourth immunisation. Two sera from the 30 µg R0.6C/AlOH + Matrix M, 100 µg R0.6C/AlOH and 100 µg R0.6C/AlOH + Matrix M arms were selected based on anti-6C antibody concentrations (ranging 7.2–20.3 µg/mL). C) Correlation between anti-6C IgG serum concentrations and transmission-reducing activity of the concentrated IgGs purified therefrom (Spearman's ρ= 1.00, ρ= 0.0028). Dashed lines at 80% indicate the predefined efficacy threshold of interest.[4,26] Asterisks indicate TRA is statistically significantly higher than the threshold of 80%.

Discussion

In this first-in-human study of a Pfs48/45-based *P. falciparum* transmission-blocking vaccine we show that vaccination with R0.6C/AlOH with or without Matrix-M is safe, immunogenic and induces functional Pfs48/45-specific transmission-blocking antibodies. While participants' sera did not directly achieve TRA, concentrated anti-R0.6C IgGs purified from selected participants' sera following vaccination exhibited strong TRA in SMFA. Together these data prove the concept of a 6C-based vaccine, but show that induced serum antibody concentrations were too low to directly confer TRA.

Although vaccination with R0.6C was generally well tolerated, the addition of Matrix-M to the R0.6C/Alhydrogel formulation induced more pronounced local reactogenicity, a finding that is likely associated with this adjuvant's enhancing effect on R0.6C immunogenicity. As observed in pre-clinical rodent studies, the addition of a second adjuvant Matrix-M to the vaccine formulation significantly increased R0.6C and 6C antibody concentrations.[16] Moreover, in contrast to R0.6C/AlOH alone, antibody responses induced by R0.6C/AlOH + Matrix-M peaked after already the second vaccine dose rather than the fourth. However, although anti-6C IgG concentrations induced by R0.6C/AlOH + Matrix-M were still significantly higher than those induced by R0.6C/AlOH alone, anti-6C IgG as a fraction of total anti-R0.6C IgG was relatively lower in R0.6C/AlOH + Matrix-M sera, suggesting that the addition of Matrix-M adjuvant preferentially favours responses to the GLURP R0 fragment, which has been shown to be immunodominant in other fusion-protein vaccines.[27]

It can be inferred that the addition of Matrix-M effectively accelerates the response to R0.6C, rapidly reaching a saturation point unattainable without Matrix-M; this response nevertheless remains insufficient to directly exert TRA, suggesting a likely impediment within the R0.6C construct itself. Furthermore, in contrast to several other malaria vaccines,[28-31] the delayed fourth dose did not significantly increase vaccine-specific IgG responses in the R0.6C/AlOH with Matrix-M study arms. While we cannot rule out that subsequent vaccine doses with either adjuvant combination may have resulted in enhanced affinity maturation, it remains a valid consideration that such maturation could have been inadequate or targeted towards non-protective epitopes of 6C. Consequently, this might have led to an inability to elicit a directly measurable functional response in sera.

There are a number of interesting observations in our current vaccine trial that are of relevance for future vaccine optimization. Firstly, IgG responses against 6C made up only 10–40% of total IgG responses against R0.6C, indicating that the greatest fraction of induced antibodies target the non-functional R0 fragment. The fraction of 6C/R0.6C IgGs was roughly concordant with the relative size of the 6C fragment compared to the overall R0.6C fusion protein.[17] Secondly, the modest antibody responses were not dependent on the vaccine dose in the tested dose range, as antibody concentrations resulting from 30 µg and 100 µg R0.6C vaccine doses with a given adjuvant combination were similar. A clinical trial involving TB31F, a potent humanised monoclonal antibody that binds a conserved epitope on the 6C fragment of Pfs48/45, was recently conducted.[12] The study showed that the concentration of TB31F reaching 80% TRA, a threshold historically used to support clinical development of TBV, was determined to be 2.1 µg/mL (95% CI 1.9–2.3).[14] This is lower than the anti-6C IgG concentrations that were induced in our current study (geometric mean 5.4 µg/mL in Matrix-M groups, SD 6.0). A likely explanation for this discrepancy could be that the total anti-6C IgG concentrations measured in our sera represent a polyvalent response of antibodies with different potencies, as we have previously identified human mAbs against 6C that differ in potency.[10] Compared with other subunit vaccines, where antigen-specific antibody concentrations of > 100 µg/mL are not exceptional,[32,33] the anti 6C-antibody levels induced in this study are modest.

Our functional results contrast with findings in pre-clinical animal models. Whereas we observed functional activity only using concentrated IgG but not directly in human sera, antibody responses in (unconcentrated) sera from animals were sufficient to induce > 99% TRA in SMFA.[15,34] This discordance may be attributed to several factors, including interspecies differences in the immune system and immunological experience of a human adult compared with a laboratory mouse. Additionally, disparities in antigen presentation between animal models and humans may underlie differences in the quality and specificity of the elicited antibody responses.[35,36] Nevertheless, our results suggest that up to 1–2 orders of magnitude higher anti-6C titres would be needed to achieve substantial transmission-reducing activity. Immunisations with an optimised vaccine formulation might achieve such sufficiently high anti-6C antibody concentrations. A more immunogenic vaccine delivery platform could be assessed, such as an mRNA vaccine[37,38] or virus-like particles, as these were shown to enhance antibody responses in mice against the TBV candidate Pfs25.[39,40] Additionally, evaluation of R0.6C in an endemic

setting may demonstrate superior antibody induction due to boosting of naturally acquired antibodies.

The greatest part of the total IgG response against R0.6C targets the non-functional R0 domain, which was included in the R0.6C construct to achieve the correct conformation of 6C during expression in *L. lactis*. Replacing the R0 domain with another 6C-stabilising component that is either less immunodominant, or that itself also induces transmission-blocking antibodies, could thus be beneficial. One promising candidate is the Pro domain of Pfs230, another antigen known to be the target of both naturally occurring and vaccine-induced transmission-blocking antibodies.[41] The functional domains of Pfs230 and Pfs48/45 have been fused with a linker sequence derived from CSP in the ProC6C construct, which was shown to elicit high titres of functional transmission-blocking antibodies in mice.[34] Two phase 1 trials are currently evaluating the safety and immunogenicity of this TBV candidate,[42] adsorbed to AlOH and formulated with and without Matrix-M adjuvant, in malaria-endemic populations in Burkina Faso (PACTR202201848463189) and Mali (ISRCTN13649456). An alternative strategy is to enhance the stability and biophysical properties of Pfs48/45-6C by combinatorial structure-based engineering of the Pfs48/45 antigen, to better focus the immune response against protective epitopes. This approach has been demonstrated to increase the transmission inhibitory capacity of sera by 1–2 orders of magnitude in rodents across three vaccine platforms, compared to the wild-type antigen,[43] although this has not yet been assessed in humans.

Furthermore, it should be noted that it remains uncertain how TRA, as measured in SMFA, relates to true transmission reduction under field conditions. Due to its relatively high numbers of gametocytes in each blood meal that need to be neutralized, the SMFA might be too stringent, leading to an underestimation of the vaccine efficacy.

This study has several limitations. Firstly, the sample size was small and the study was not blinded. Although this is common in phase 1 trials, it is conceivable that this may have biased the reporting of adverse events. Secondly, the study was carried out during the SARS-CoV-2 pandemic; as a consequence, concurrent (suspected) SARS-CoV-2 infections led to postponement of follow-up visits outside the protocol-defined window on 7 occasions and to minor postponement of the third or fourth vaccination in four participants. The impact of postponing these follow-up visits was considered

to have negligible impact on the safety and immunogenicity. In all instances, the greatest post-vaccination 'risk-window' had already passed, and the relatively short delay, when viewed in the context of the overall immunisation schedule timelines, is expected to exert, at most, a minor and immunologically insignificant impact thereon.

Conclusions

We conclude that vaccination with R0.6C/AlOH with or without Matrix-M is safe and immunogenic, but the induced serum antibody titres were insufficient to achieve the threshold for *P. falciparum* transmission reduction in a malaria naïve population. Purified and concentrated anti-R0.6C IgGs were nevertheless able to induce up to 99% TRA, demonstrating for the first time the functionality and transmission-blocking potential of antibodies induced by a Pfs48/45-based vaccine.

Availability of data and materials

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Contributions

MA, JP, MT, MMJ, MBBM conceived the study and developed the study protocol. MMJ and MBBM provided oversight and supervision. MA, MJS, and KT2 did the

ELISAs. CMM and MMJ did the antibody assays. GJG and MVB did the standard membrane feeding assay. MA did the statistical analysis. BGM provided clinical and statistical advice. MA, MJS, KT1, KT2, and MBBM coordinated the trial and oversaw data collection and management. MA, MJS, CMM, KT1, KT2, GJG, MVB, JMR, KLB, TB, MMJ, MBBM and BGM accessed and verified the data. MA, MJS, CMM, RWS, TB, MMJ, and MBBM analysed the data, and MA, MJS, CMM, MMJ, and MBBM wrote the original draft of the manuscript. All authors had access to the pseudonymized raw data and agreed to the submission for publication. All authors read and approved the final manuscript.

References

1. Blagborough AM, Churcher TS, Upton LM, Ghani AC, Gething PW, Sinden RE. Transmission-blocking interventions eliminate malaria from laboratory populations. Nat Commun 2013; 4(1): 1812.
2. Rabinovich RN, Drakeley C, Djimde AA, Hall BF, Hay SI, Hemingway J, et al. malERA: an updated research agenda for malaria elimination and eradication. PLoS Med 2017; 14(11): e1002456.
3. Griffin JT, Hollingsworth TD, Okell LC, Churcher TS, White M, Hinsley W, et al. Reducing Plasmodium falciparum malaria transmission in Africa: a model-based evaluation of intervention strategies. PLoS Med 2010; 7(8): e1000324.
4. Sauerwein RW, Bousema T. Transmission blocking malaria vaccines: assays and candidates in clinical development. Vaccine 2015; 33(52): 7476–82.
5. Targett GA, Greenwood BM. Malaria vaccines and their potential role in the elimination of malaria. Malar J 2008; 7(1): S10.
6. Duffy PE. Transmission-blocking vaccines: harnessing herd immunity for malaria elimination. Expert Rev Vaccines 2021; 20(2): 185–98.
7. Healy SA, Anderson C, Swihart BJ, Mwakingwe A, Gabriel EE, Decederfelt H, et al. Pfs230 yields higher malaria transmission-blocking vaccine activity than Pfs25 in humans but not mice. J Clin Investig 2021; 131(7): e146221.
8. Sagara I, Healy SA, Assadou MH, Kone M, Swihart BJ, Kwan JL, et al. Malaria transmission-blocking vaccines Pfs230D1-EPA and Pfs25-EPA in Alhydrogel in healthy Malian adults; a phase 1, randomised, controlled trial. Lancet Infect Dis 2023; 23(11): 1266–79.
9. van Dijk MR, Janse CJ, Thompson J, Waters AP, Braks JA, Dodemont HJ, et al. A central role for P48/45 in malaria parasite male gamete fertility. Cell 2001; 104(1): 153–64.
10. Fabra-Garcia A, Hailemariam S, de Jong RM, Janssen K, Teelen K, van de Vegte-Bolmer M, et al. Highly potent, naturally acquired human monoclonal antibodies against Pfs48/45 block Plasmodium falciparum transmission to mosquitoes. Immunity 2023; 56(2): 406–19 e7.
11. Stone WJR, Campo JJ, Ouédraogo AL, Meerstein-Kessel L, Morlais I, Da D, et al. Unravelling the immune signature of Plasmodium falciparum transmission-reducing immunity. Nat Commun 2018; 9(1): 558.
12. Kundu P, Semesi A, Jore MM, Morin MJ, Price VL, Liang A, et al. Structural delineation of potent transmission-blocking epitope I on malaria antigen Pfs48/45. Nat Commun 2018; 9(1): 4458.
13. Outchkourov N, Vermunt A, Jansen J, Kaan A, Roeffen W, Teelen K, et al. Epitope analysis of the malaria surface antigen pfs48/45 identifies a subdomain that elicits transmission blocking antibodies. J Biol Chem 2007; 282(23): 17148–56.
14. van der Boor SC, Smit MJ, van Beek SW, Ramjith J, Teelen K, van de Vegte-Bolmer M, et al. Safety, tolerability, and Plasmodium falciparum transmission-reducing activity of monoclonal antibody TB31F: a single-centre, open-label, first-in-human, dose-escalation, phase 1 trial in healthy malaria-naive adults. Lancet Infect Dis 2022; 22(11): 1596–605.
15. Singh SK, Roeffen W, Mistarz UH, Chourasia BK, Yang F, Rand KD, et al. Construct design, production, and characterization of Plasmodium falciparum 48/45 R0.6C subunit protein produced in Lactococcus lactis as candidate vaccine. Microb Cell Fact 2017; 16(1): 97.

16. Singh SK, Plieskatt J, Chourasia BK, Fabra-García A, Garcia-Senosiain A, Singh V, et al. A reproducible and scalable process for manufacturing a Pfs48/45 based Plasmodium falciparum transmission-blocking vaccine. Front Immunol 2021; 11: 606266.
17. Singh SK, Roeffen W, Andersen G, Bousema T, Christiansen M, Sauerwein R, Theisen M. A Plasmodium falciparum 48/45 single epitope R0.6C subunit protein elicits high levels of transmission blocking antibodies. Vaccine 2015; 33(16): 1981–6.
18. Singh SK, Thrane S, Chourasia BK, Teelen K, Graumans W, Stoter R, et al. Pfs230 and Pfs48/45 fusion proteins elicit strong transmission-blocking antibody responses against Plasmodium falciparum. Front Immunol 2019; 10: 1256.
19. Reimer JM, Karlsson KH, Lövgren-Bengtsson K, Magnusson SE, Fuentes A, Stertman L. Matrix-M™ adjuvant induces local recruitment, activation and maturation of central immune cells in absence of antigen. PLoS One 2012; 7(7): e41451.
20. Magnusson SE, Altenburg AF, Bengtsson KL, Bosman F, de Vries RD, Rimmelzwaan GF, Stertman L. Matrix-M™ adjuvant enhances immunogenicity of both protein- and modified vaccinia virus Ankara-based influenza vaccines in mice. Immunol Res 2018; 66(2): 224–33.
21. Food and Drug Administration CfBEaR. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. 2007.
22. Singh SK, Thrane S, Janitzek CM, Nielsen MA, Theander TG, Theisen M, et al. Improving the malaria transmission-blocking activity of a Plasmodium falciparum 48/45 based vaccine antigen by SpyTag/SpyCatcher mediated virus-like display. Vaccine 2017; 35(30): 3726–32.
23. Clatworthy AJ, Jones LV, Whitehouse MJ. The gas chromatography mass spectrometry of the major metabolites of flurazepam. Biomed Mass Spectrom 1977; 4(4): 248–54.
24. van der Kolk M, De Vlas SJ, Saul A, van de Vegte-Bolmer M, Eling WM, Sauerwein RW. Evaluation of the standard membrane feeding assay (SMFA) for the determination of malaria transmission-reducing activity using empirical data. Parasitology 2005; 130 (Pt 1): 13–22.
25. Ramjith J, Alkema M, Bradley J, Dicko A, Drakeley C, Stone W, Bousema T. Quantifying reductions in Plasmodium falciparum infectivity to mosquitos: a sample size calculator to inform clinical trials on transmission-reducing interventions. Front Immunol 2022; 13: 899615.
26. Churcher TS, Blagborough AM, Delves M, Ramakrishnan C, Kapulu MC, Williams AR, et al. Measuring the blockade of malaria transmission–an analysis of the standard membrane feeding assay. Int J Parasitol 2012; 42(11): 1037–44.
27. Esen M, Kremsner PG, Schleucher R, Gassler M, Imoukhuede EB, Imbault N, et al. Safety and immunogenicity of GMZ2 - a MSP3-GLURP fusion protein malaria vaccine candidate. Vaccine 2009; 27(49): 6862–8.
28. Moon JE, Greenleaf ME, Regules JA, Debois M, Duncan EH, Sedegah M, et al. A phase IIA extension study evaluating the effect of booster vaccination with a fractional dose of RTS, S/AS01(E) in a controlled human malaria infection challenge. Vaccine 2021; 39(43): 6398–406.
29. Moon JE, Ockenhouse C, Regules JA, Vekemans J, Lee C, Chuang I, et al. A phase IIa controlled human malaria infection and immunogenicity study of RTS, S/AS01E and RTS, S/AS01B delayed fractional dose regimens in malaria-naive adults. J Infect Dis 2020; 222(10): 1681–91.

30. Regules JA, Cicatelli SB, Bennett JW, Paolino KM, Twomey PS, Moon JE, et al. 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 2016; 214(5): 762–71.
31. Minassian AM, Silk SE, Barrett JR, Nielsen CM, Miura K, Diouf A, et al. Reduced blood-stage malaria growth and immune correlates in humans following RH5 vaccination. Med 2021; 2(6): 701–19.e19.
32. Demonbreun AR, Sancilio A, Velez MP, Ryan DT, Saber R, Vaught LA, et al. Comparison of IgG and neutralizing antibody responses after one or two doses of COVID-19 mRNA vaccine in previously infected and uninfected individuals. EClinicalMedicine 2021; 38: 101018.
33. Keshavarz B, Richards NE, Workman LJ, Patel J, Muehling LM, Canderan G, et al. Trajectory of IgG to SARS-CoV-2 after vaccination with BNT162b2 or mRNA-1273 in an employee cohort and comparison with natural infection. Front Immunol 2022; 13: 850987.
34. Singh SK, Plieskatt J, Chourasia BK, Singh V, Bengtsson KL, Reimer JM, et al. Preclinical development of a Pfs230-Pfs48/45 chimeric malaria transmission-blocking vaccine. npj Vaccines 2021; 6(1): 120.
35. Simwela NV, Waters AP. Current status of experimental models for the study of malaria. Parasitology 2022; 149(6): 1–22.
36. Langhorne J, Buffet P, Galinski M, Good M, Harty J, Leroy D, et al. The relevance of non-human primate and rodent malaria models for humans. Malar J 2011; 10: 23.
37. Laurens MB. Novel malaria vaccines. Hum Vaccin Immunother 2021; 17(11): 4549–52.
38. Dolgin E. How COVID unlocked the power of RNA vaccines. Nature 2021; 589(7841): 189–91.
39. Thrane S, Janitzek CM, Matondo S, Resende M, Gustavsson T, de Jongh WA, et al. Bacterial superglue enables easy development of efficient virus-like particle based vaccines. J Nanobiotechnology 2016; 14: 30.
40. Brune KD, Leneghan DB, Brian IJ, Ishizuka AS, Bachmann MF, Draper SJ, et al. Plug-and-display: decoration of virus-like particles via isopeptide bonds for modular immunization. Sci Rep 2016; 6: 19234.
41. de Jong RM, Tebeje SK, Meerstein-Kessel L, Tadesse FG, Jore MM, Stone W, Bousema T. Immunity against sexual stage Plasmodium falciparum and Plasmodium vivax parasites. Immunol Rev 2020; 293(1): 190–215.
42. Sauerwein RW, Plieskatt J, Theisen M. 40 Years of Pfs48/45 research as a transmission-blocking vaccine target of Plasmodium falciparum malaria. Am J Trop Med Hyg 2022; 107(Suppl 3): 22–6.
43. McLeod B, Mabrouk MT, Miura K, Ravichandran R, Kephart S, Hailemariam S, et al. Vaccination with a structure-based stabilized version of malarial antigen Pfs48/45 elicits ultra-potent transmission-blocking antibody responses. Immunity 2022; 55(9): 1680–92.e8.

Supplementary Material

Please scan the QR code below to access the supplementary material for Chapter 5:

CHAPTER 6

Safety, tolerability, and *Plasmodium falciparum* transmission-reducing activity of monoclonal antibody TB31F: a single-centre, open-label, first-in-human, dose-escalation, phase 1 trial in healthy malaria-naive adults

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Summary

Background

Malaria elimination requires interruption of the highly efficient transmission of *Plasmodium* parasites by mosquitoes. TB31F is a humanised monoclonal antibody that binds the gamete surface protein Pfs48/45 and inhibits fertilisation, thereby preventing further parasite development in the mosquito midgut and onward transmission. We aimed to evaluate the safety and efficacy of TB31F in malaria-naive participants.

Methods

In this open-label, first-in-human, dose-escalation, phase 1 clinical trial, healthy, malaria-naive, adult participants were administered a single intravenous dose of 0·1, 1, 3, or 10 mg/kg TB31F or a subcutaneous dose of 100 mg TB31F, and monitored until day 84 after administration at a single centre in the Netherlands. The primary outcome was the frequency and magnitude of adverse events. Additionally, TB31F serum concentrations were measured by ELISA. Transmission-reducing activity (TRA) of participant sera was assessed by standard membrane feeding assays with *Anopheles stephensi* mosquitoes and cultured *Plasmodium falciparum* gametocytes. The trial is registered with Clinicaltrials.gov, NCT04238689.

Findings

Between Feb 17 and Dec 10, 2020, 25 participants were enrolled and sequentially assigned to each dose (n=5 per group). No serious or severe adverse events occurred. In total, 33 grade 1 and six grade 2 related adverse events occurred in 20 (80%) of 25 participants across all groups. Serum of all participants administered 1 mg/kg, 3 mg/kg, or 10 mg/kg TB31F intravenously had more than 80% TRA for 28 days or more, 56 days or more, and 84 days or more, respectively. The TB31F serum concentration reaching 80% TRA was 2·1 µg/mL (95% CI 1·9–2·3). Extrapolating the duration of TRA from antibody kinetics suggests more than 80% TRA is maintained for 160 days (95% CI 136–193) following a single intravenous 10 mg/kg dose.

Interpretation

TB31F is a well tolerated and highly potent monoclonal antibody capable of completely blocking transmission of *P falciparum* parasites from humans to mosquitoes. In areas of seasonal transmission, a single dose might cover an entire malaria season.

Research in context

Evidence before this study

Malaria is a global health priority with over 200 million cases and over 500 000 deaths that result primarily from *Plasmodium falciparum* infections. In addition to strategies focusing on preventing and treating individual cases of malaria, interrupting the highly efficient transmission of malaria is key to reducing the global burden of this disease. We searched PubMed on March 8, 2022, for clinical trials testing transmission blocking vaccines and monoclonal antibodies against *P falciparum* malaria, using the search terms: (falciparum OR malaria) AND ((transmission blocking) OR (transmission-blocking) OR (transmission reducing) OR (transmission-reducing)) AND (vaccine OR monoclonal). No language or date restrictions were applied. No records were present of clinical trials involving transmission-blocking monoclonal antibodies; although, one study reports the clinical testing of a monoclonal antibody that targets the pre-erythrocytic stage of the parasite and hence can prevent infection. Among transmission-blocking monoclonal antibodies evaluated to date in preclinical studies, the humanised antibody TB31F has shown the most potent functional activity.

Added value of this study

This is the first clinical trial assessing the safety and efficacy of a *P falciparum* transmission blocking monoclonal antibody (TB31F). We report TB31F administration to malaria-naive healthy participants to be safe and well tolerated at all doses tested. TB31F in participants' serum showed potent activity, reaching the benchmark of at least 80% reduction in transmission of parasites to mosquitoes in the standard membrane feeding assay at serum concentrations as low as 2·1 µg/mL. Moreover, such activity was estimated to last for up to 5 months after a single administration.

Implications of all the available evidence

The preliminary safety and highly promising efficacy profile of TB31F, in particular its ability to potently block transmission of *P falciparum* parasites to mosquitoes, underscore its clinical potential. Current malaria control measures, even when complemented by the widespread deployment of the pre-erythrocytic malaria vaccine RTS,S/AS01, as recommended by WHO in October, 2021, will be insufficient to prevent all episodes of malaria and subsequent onward transmission of parasites. TB31F represents a promising complementary tool to be deployed in several use-scenarios,

including in areas of seasonal malaria transmission, where a single administration might have transmission-blocking activity for the duration of an entire season.

Introduction

Malaria is a global health priority with over 200 million yearly cases worldwide, including more than half a million deaths resulting primarily from *Plasmodium falciparum* infections.[1] Malaria infections and deaths have been rising over the past five years.[1] The emergence and spread of artemisinin resistance in Africa warns that gains in reducing malaria burden, which were attributed in part to access to efficacious artemisinin-based treatment, are under threat.[2,3]

In 2021, landmark progress was achieved in the field of malaria vaccines. WHO issued a recommendation for widespread use of RTS,S/AS01, the world's first approved malaria vaccine, among children living in regions with moderate to high *P falciparum* malaria transmission. Despite its important value in reducing malaria morbidity and mortality, deployment of RTS,S/AS01 will be insufficient to prevent all episodes of malaria. Moreover, this vaccine does not directly prevent onward transmission of parasites to mosquitoes and remains susceptible to selection and spread of escape mutants. The high efficacy of monoclonal antibody CIS43LS in preventing infections in a human challenge model underscores the clinical potential of monoclonal antibodies as a complementary tool to combat malaria, as shown for other infectious diseases.[4–7]

Malaria transmission depends on the uptake of male and female gametocytes by blood feeding *Anopheles* mosquitoes in whose midgut gametocytes activate into gametes, fertilise, and ultimately render the mosquito infectious to the next human host. The long duration of (asymptomatic) *P falciparum* infections in humans, the abundance of mosquitoes that bite multiple hosts, and efficient transmission of parasites from humans to mosquitoes and vice versa, all contribute to reproduction rates (R_0) for malaria that exceed 100 in many African settings.[8]

Malaria transmission-blocking vaccines aim to induce antibodies that target the surface of sexual stage parasites and are coingested with gametocytes during the mosquito bloodmeal, consequently preventing parasite development

in the mosquito midgut and hence onward transmission.[9] The leading transmission-blocking vaccine targets are Pfs48/45 and Pfs230, two surface proteins expressed on *P falciparum* gametocytes and early gametes, that are being evaluated in phase 1 (NCT04862416) and phase 2 (NCT03917654) clinical trials, respectively. The effect of these vaccines will depend on their capacity to induce and maintain effective antibody titres against specific key epitopes,[10] whereby the titre required for functional activity is target dependent and antibody dependent. The most potent transmission-blocking antibody described to date is the rat monoclonal antibody 85RF45.1, which was derived from rats immunised with *P falciparum* NF54 gametocytes.[11] Antibody 85RF45.1 targets a highly conserved epitope on Pfs48/45 and blocks onward transmission of genetically diverse parasites to other humans.[9,11–13] This monoclonal antibody has been humanised as TB31F, which maintains the original binding characteristics and potency.[12] A safe and highly efficacious transmission-blocking monoclonal antibody for administration in humans, when used in combination with other malaria control measures, would be a valuable tool for malaria elimination. Here, we report the safety, tolerability, pharmacokinetics, and functional transmission-reducing activity (TRA) of the humanised monoclonal antibody TB31F.

Methods

Study design and participants

This single-centre, open-label, first-in-human, dose-escalation, phase 1 clinical study evaluating the safety and efficacy of humanised monoclonal antibody TB31F (supplementary appendix file 1) was done at the Radboud University Medical Center (Radboudumc) in Nijmegen, the Netherlands, between Jan 23, 2020, and March 4, 2021. The study received approval from the Arnhem–Nijmegen Committee on Research Involving Human Subjects (NL69779.091.19). The study was done in accordance with the latest Fortaleza revision of the Declaration of Helsinki (2013), the Netherlands' Medical Research Involving Human Subjects Act, ICH Good Clinical Practice standards, and local regulatory requirements. The study population was composed of healthy adults aged 18–35 years who are malaria-naive. Upon informed written consent, participants were screened for eligibility as described in the protocol.

Study Procedures

25 eligible participants were included and upon enrolment sequentially allocated from group 1 to group 5 (n=5 per group; figure 1). Participants in groups 1–4 received 0·1, 1, 3, and 10 mg/kg humanised monoclonal antibody TB31F by intravenous infusion, respectively. Group 5 participants (n=5) received 100 mg TB31F subcutaneously. TB31F administration took place in the medium care unit under direct clinical supervision. Before administration, all participants received premedication consisting of 1000 mg paracetamol orally and 2 mg clemastine intravenously as a safety precaution. TB31F was administered via slow intravenous drip to participants of groups 1–4, at increasing infusion rates per dose group (supplementary table 1 and 2) as an additional precautionary measure against infusion reactions. TB31F was administered subcutaneously (abdomen) to participants of group 5, divided equally in volumes of 1 mL between two injection sites.

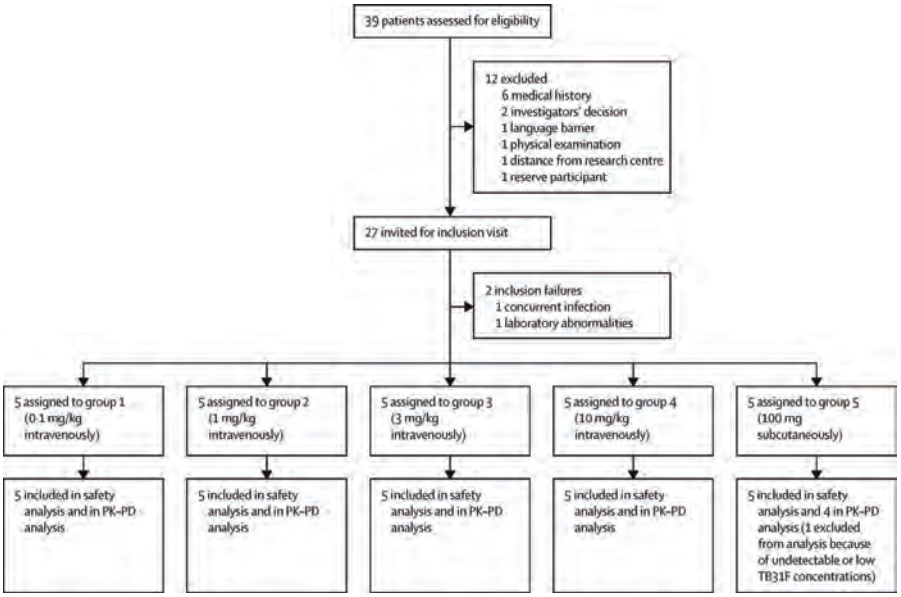

Figure 1. Study profile. PK–PD analysis=pharmacokinetic and pharmacodynamic analysis.

On the day of administration, collection of vital signs, safety data, and serum for pharmacokinetic and pharmacodynamic measurements was done before infusion or administration, upon end of infusion or administration, and 1, 3, and 6 h after end of infusion or administration. All participants were observed for 6 h after end of infusion or administration. Escalation to the next higher dose

group was dependent on the absence of safety signals and on positive approval by an independent safety monitoring committee.

Follow-up visits were done on days 1, 2, 7, 14, 21, 28, 56, and 84 after TB31F administration. Group 5 participants were additionally seen on days 4 and 10 after administration to detect peak TB31F concentrations. During follow-up visits, clinical safety data and blood samples for safety, pharmacokinetic, and pharmacodynamic analyses were collected. Due to COVID-19-related restrictions in force at the time, the routine follow-up visit scheduled 56 days after administration could not be done for group 1 participants.

Outcomes

Primary outcomes included the number and severity of solicited local and systemic adverse events, unsolicited adverse events, serious adverse events, and clinically significant laboratory abnormalities. Secondary outcomes included serum TB31F monoclonal antibody concentration over time to determine pharmacokinetic parameters, and functional TRA as assessed by standard membrane feeding assay (SMFA).

Adverse events were recorded and graded by the attending clinical investigator as mild (grade 1; easily tolerated), moderate (grade 2; interferes with daily activity) or severe (grade 3; prevents normal activity; appendix pp 12–13, and in the protocol). All participants received a memory aid booklet to register symptoms throughout the study and an oral thermometer to register their daily body temperature for 6 consecutive days after TB31F administration. Solicited local adverse events were defined as pain, redness, and swelling at the injection site up to 7 days after TB31F administration. Solicited systemic adverse events were defined as fever, headache, myalgia, fatigue, chills, and rash up to 28 days after TB31F administration. Unsolicited and serious adverse events were recorded through to the end of the study (day 84). Clinical laboratory results assessed as adverse events were scored for severity with the adapted Food and Drug Administration Toxicity Tables. Antidrug antibody levels in participants were assessed by sandwich ELISA at the Human Immunology Laboratory in London, UK, as an exploratory outcome (supplementary figure 2).

Serum concentrations of TB31F were measured by ELISA against the recombinant protein R0·6C, which contains the 6C fragment of the Pfs48/45 antigen to which TB31F binds.[14] Antibody concentrations were quantified

with ADAMSEL software (version 1.1) with a TB31F standard curve and three duplicate serum dilutions. The limit of detection was determined by the serum dilutions tested, and therefore, differed per group; the detection limit was 0·039 µg/mL for group 1 (0·1 mg/kg intravenously), 0·39 µg/mL for group 2 (1 mg/kg intravenously), 1·17 µg/mL for group 3 (3 mg/kg intravenously), 3·91 µg/mL for group 4 (10 mg/kg intravenously), and 0·39 µg/mL for group 5 (100 mg subcutaneously) samples.

For the intravenous and subcutaneous groups, the following pharmacokinetic parameters were determined by non-compartmental analysis for each individual curve: the maximum concentration (C_{max}), the area under the concentration-time curve from 0 to 12 weeks, and from time zero to infinity with the trapezoidal rule, and the elimination half-life. The apparent clearance and volume of distribution were additionally determined for individuals receiving intravenous TB31F administration. The bioavailability and the time of C_{max} (T_{max}) were additionally determined for individuals in the subcutaneous administration group. Projected TB31F concentrations beyond day 84 were estimated for each participant with concentrations from day 7 to day 84 after administration assuming first-order kinetics.

TRA was determined at predefined intervals by SMFA with participants' sera.[15] In short, 90 µL of participant's serum was mixed with 150 µL packed red blood cells and cultured *P falciparum* NF54 gametocytes, and 30 µL naive human serum containing active complement, before feeding to *Anopheles stephensi* (Sind-Kasur Nijmegen strain) mosquitoes. Time series of individual participants were grouped in the same SMFA experiment. Additional SMFA with sera from participants receiving 10 mg/kg TB31F was done as a post-hoc analysis to determine TRA against the genetically distant Asian *P falciparum* isolate NF135.[16] TRA was expressed as the reduction of oocyst count in mosquitoes fed on gametocytes in the presence of participants' serum (or purified IgG) compared with mosquitoes fed on gametocytes in the presence of pooled naive serum (or IgG).[15,17] Given the lower precision of low TRA estimates and the historical threshold value of more than 80% TRA to support clinical development of transmission-blocking vaccines, TRA of more than 80% was predefined as the efficacy threshold of interest.[18,19]

Statistical analysis

Study sample size was chosen pragmatically on the basis of the assessment of safety and in line with similar first-in-human dose-escalation trials. The

study's original sample size of 20 (four intravenous groups of five participants each) was determined to allow a power of 90% to observe at least one event if the true rate of such an event is 10·9% or more; and a 90% chance of observing no events if the true rate is 0·5% or less. The fifth (subcutaneous) group was added later and pragmatically included the same number of participants as the other groups. Safety data are shown as frequencies and percentages are tabulated. TB31F pharmacokinetic parameters were assessed by standard non-compartmental methods, with multiple observations per group and per study participant. TB31F serum concentration (ie, concentration in the total liquid volume of the bloodmeal) at which 80% TRA is expected (IC_{80}) was estimated by linear regression, regressing the square root of serum concentration on the log-mean oocyst ratio[17]–ie,

$$IC80 = \left(\frac{\left(\log\left(\frac{100}{100-80}\right)-\hat{\beta}_0\right)}{\hat{\beta}_1}\right)^2$$

where β_0 and β_1 are the estimated regression coefficients for the intercept and slope, respectively. The delta method was used to calculate 95% CIs for the IC_{80}.

Safety data analysis was done in IBM SPSS Statistics (version 25.0.0.1) and Prism 9 (version 9.2.0). SMFA data analysis was done in R (version 4.1.1) and R studio (version 1.4.1717). Pharmacokinetic parameters were determined with non-compartmental analysis in Phoenix WinNonlin (version 8.3). The trial is registered with ClinicalTrials.gov, NCT04238689.

Role of the funding source

This study was funded by PATH's Malaria Vaccine Initiative, Washington, DC, USA. The funder was involved in study design, data collection, analysis, and interpretations, and contributed to the writing of the report.

Results

From Jan 23 to Dec 8, 2020, 39 participants were screened for eligibility. In total, 25 participants were enrolled between Feb 17 and Dec 10, 2020 (figure 1). Baseline characteristics were similar between the five study groups (table 1). Overall, 13 (52%) of 25 participants were women. The mean age was 23·5 years (range 19·0–34·0), the mean bodyweight was 74·2 kg (range 53·0–93·0),

and the mean body-mass index was 24·1 kg/m^2 (range 19·5–29·5). All participants remained in follow-up until the end of the study and were included in the safety analysis.

	Group 1 (n=5)	Group 2 (n=5)	Group 3 (n=5)	Group 4 (n=5)	Group 5 (n=5)	Total (n=25)
Sex						
Women	3 (60%)	3 (60%)	2 (40%)	4 (80%)	1 (20%)	13 (52%)
Men	2 (40%)	2 (40%)	3 (60%)	1 (20%)	4 (80%)	12 (48%)
Age, years	22·0 (21·0–24·0)	24·8 (22·0–26·0)	26·8 (24·0–34·0)	21·6 (19·0–25·0)	22·2 (19·0–25·0)	23·5 (19·0–34·0)
Weight, kg	73·6 (60·4–84·8)	75·5 (68·8–91·2)	72·0 (53·0–86·0)	71·5 (63·2–91·8)	78·4 (63.2–93·0)	74·2 (53·0–93·0)
Body-mass index, kg/m^2	24·3 (19·5–29·5)	25·0 (22·0–27·8)	23·4 (21·6–26·4)	23·9 (21·7–29·0)	23·9 (21·7–25·0)	24·1 (19·5–29·5)

Table 1. Baseline characteristics. Data are n (%) or mean (range).

Both intravenous and subcutaneous administrations of TB31F were well tolerated. No serious or grade 3 (severe) adverse events occurred. Furthermore, no infusion reactions occurred and no dose adjustments or temporary interruptions of TB31F administration were necessary. Overall, 20 (80%) of 25 participants had at least one adverse event that was possibly, probably, or definitely related to TB31F administration. Most solicited local adverse events took place in the subcutaneous group in which four (80%) of five participants had grade 1 (mild) pain of short duration (≤2 min) during subcutaneous administration (table 2, supplementary table 6). 13 (52%) of 25 participants had fatigue, which was the most common solicited systemic adverse event. Most of these episodes occurred on the day of TB31F administration. Additionally, one otherwise asymptomatic participant reported a grade 1 fever (38·1°C) on the day of TB31F administration, which resolved the same day and was assessed as possibly related to study product administration. Our trial was not powered to allow meaningful comparisons of the occurrence of individual adverse events between groups. Unsolicited adverse events that occurred during the study are summarised in supplementary table 7. All adverse events are listed in supplementary table 8 and 9. No severe laboratory abnormalities occurred; all laboratory abnormalities are listed in supplementary table 10. Antidrug antibody responses were not detected in any participant at any timepoint (data not shown).

TB31F displayed dose-dependent peak concentrations and a dose-independent terminal half-life (figure 2, supplementary figure 1). Dose-proportional pharmacokinetics were observed (supplementary figure 2). Pharmacokinetic parameters, as determined by non-compartmental analysis, are summarised in supplementary table 11. TB31F administered intravenously at doses of 0·1, 1, 3, and 10 mg/kg resulted in dose-proportional maximum concentrations (C_{max}, reported as geometric mean and 95% CI) of 2·94 µg/mL (95% CI 2·34–3·69), 28·3 µg/mL (14·4–55·5), 80·8 µg/mL (55·2–118), and 255 µg/mL (177–367), respectively, at end of infusion or administration (=T_{max}). Subcutaneous administration of 100 mg TB31F resulted in a maximum concentration of 9·28 µg/mL (3·49–24·7) at day 4·3 (1·15–16·0), with a bioavailability of 52% (0·17–1·61), all reported as geometric mean and 95% CI. The overall terminal half-life was estimated to be 32·2 days.

TB31F remained detectable in serum until end of study at day 84 in all study groups, except for one participant in the subcutaneous group. In this participant, in contrast to other group 5 participants, no or very low concentrations of TB31F were detected in serum at any timepoint. Therefore, the data from this participant were only included in the safety analyses (antibody concentrations, where measurable, and TRA are provided for reference in the supplementary figure 3).

The functional activity of TB31F in participants' serum samples in reducing transmission to mosquitoes was expressed as TRA (figure 3). A single intravenous dose of 1 mg/kg, 3 mg/kg, or 10 mg/kg was sufficient to reach more than 80% TRA against the west African *P falciparum* strain NF54 for 28 days or more, 56 days or more, and 84 days or more, respectively, in all participants. A single intravenous dose of 10 mg/kg was also sufficient to maintain more than 80% TRA against the genetically distant Asian *P falciparum* isolate NF135 for 84 days or more (supplementary figure 4). Following a subcutaneous dose of 100 mg, more than 80% TRA was maintained for 28 days or more in four of five participants. The potency of administered TB31F was maintained over time (supplementary figure 5).

	Group 1 (n=5)		Group 2 (n=5)		Group 3 (n=5)		Group 4 (n=5)		Group 5 (n=5)		All (n=25)	
	Participants	Events	Participants	Events	Participants	Events	Participants	Events	Participants	Events	Participants	Events
Pain												
Grade 1	0	0	0	0	1 (20%)	1	0	0	4 (80%)	4	5 (20%)	5
Grade 2	0	0	0	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0	0	0	0
Any	0	0	0	0	1 (20%)	1	0	0	4 (80%)	4	5 (20%)	5
Redness												
Grade 1	0	0	1 (20%)	1	0	0	0	0	0	0	1 (4%)	1
Grade 2	0	0	0	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0	0	0	0
Any	0	0	1 (20%)	1	0	0	0	0	0	0	1 (4%)	1
Fever												
Grade 1	0	0	0	0	0	0	1 (20%)	1	0	0	1 (4%)	1
Grade 2	0	0	0	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0	0	0	0
Any	0	0	0	0	0	0	1 (20%)	1	0	0	1 (4%)	1
Headache												
Grade 1	1 (20%)	1	1 (20%)	1	0	0	2 (40%)	6	0	0	4 (16%)	8
Grade 2	0	0	0	0	0	0	1 (20%)	1	0	0	1 (4%)	1
Grade 3	0	0	0	0	0	0	0	0	0	0	0	0

	Group 1 (n=5)		Group 2 (n=5)		Group 3 (n=5)		Group 4 (n=5)		Group 5 (n=5)		All (n=25)	
	Participants	Events	Participants	Events	Participants	Events	Participants	Events	Participants	Events	Participants	Events
Any	1 (20%)	1	1 (20%)	1	0	0	3 (60%)	7	0	0	5 (20%)	9
Myalgia												
Grade 1	1 (20%)	1	0	0	0	0	0	0	0	0	1 (4%)	1
Grade 2	0	0	0	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0	0	0	0
Any	1 (20%)	1	0	0	0	0	0	0	0	0	1 (4%)	1
Fatigue												
Grade 1	2 (40%)	4	2 (40%)	2	1 (20%)	1	2 (40%)	2	2 (40%)	2	9 (36%)	11
Grade 2	3 (60%)	3	0	0	0	0	1 (20%)	1	0	0	4 (16%)	4
Grade 3	0	0	0	0	0	0	0	0	0	0	0	0
Any	5 (100%)	7	2 (40%)	2	1 (20%)	1	3 (60%)	3	2 (40%)	2	13 (52%)	15

Table 2. Solicited adverse events. Possibly, probably, and definitely related adverse events are reported per group. Local adverse events (pain, redness, and local swelling) are shown from administration to day 7. No local swelling was reported in any of the participants. Solicited systemic adverse events (fever, headache, myalgia, fatigue, chills, or rash) are shown from administration to day 28. No chills or rash were reported in any of the participants.

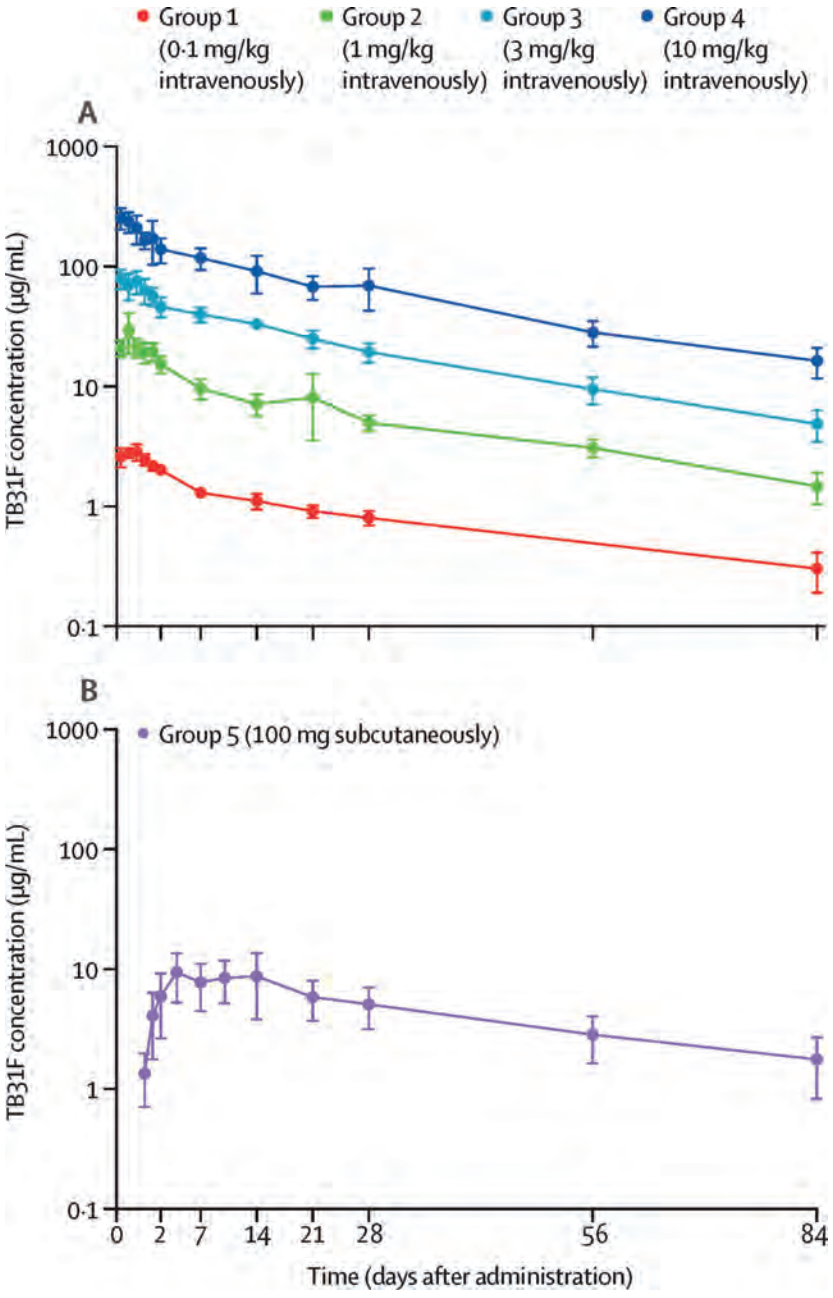

Figure 2. TB31F concentrations per dose group over time. (A) Groups 1–4 (intravenous infusion, n=5 per group). (B) Group 5 (subcutaneous administration, n=4). Datapoints and error bars represent group mean and standard deviation, respectively. For group 1, serum TB31F concentrations were unavailable for one participant at day 28 and all five participants at day 56. One participant in group 5 had no detectable or very low concentrations of TB31F at any timepoint (supplementary figure 3).

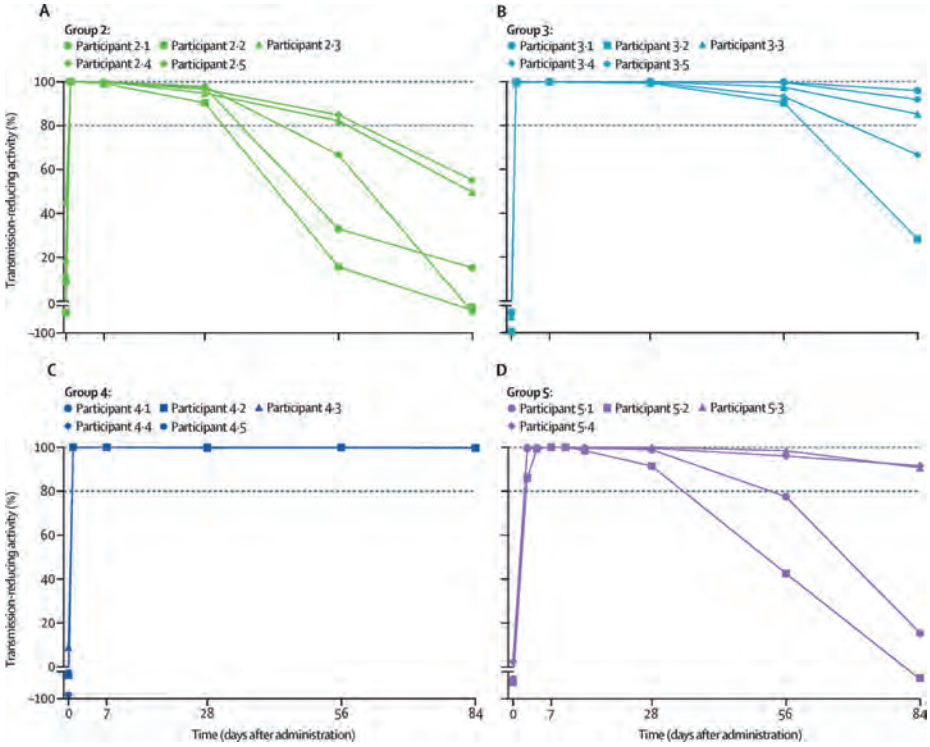

Figure 3. TRA of TB31F per dose group over time. (A) Group 2 (1 mg/kg intravenously). (B) Group 3 (3 mg/kg intravenously). (C) Group 4 (10 mg/kg intravenously). (D) Group 5 (100 mg subcutaneously). TRA was calculated by comparing the reduction of oocyst counts of participant sera compared with pooled naive serum in standard membrane feeding assay. Each line represents a participant. The horizontal dashed lines represent the 100% and 80% TRA threshold. No participants in the lowest dose group (group 1, 0·1 mg/kg) showed TRA >80% at any time point (data not shown). One participant in group 5 had no detectable or very low concentrations of TB31F, and TRA data are shown in supplementary figure 3. TRA=transmission-reducing activity.

Combining TB31F concentrations and the corresponding TRA measured in individual participants' sera at various timepoints, the concentration of TB31F reaching 80% TRA (IC_{80}) was determined to be 2·1 µg/mL (95% CI 1·9–2·3; figure 4A). Sera from participants that received 10 mg/kg TB31F (group 4) showed more than 80% TRA at the end of the follow-up (day 84). Therefore, we projected TB31F concentrations over time and extrapolated that a single intravenous dose of 10 mg/kg TB31F maintains more than 80% TRA for 160 days (95% CI 136–193; figure 4B).

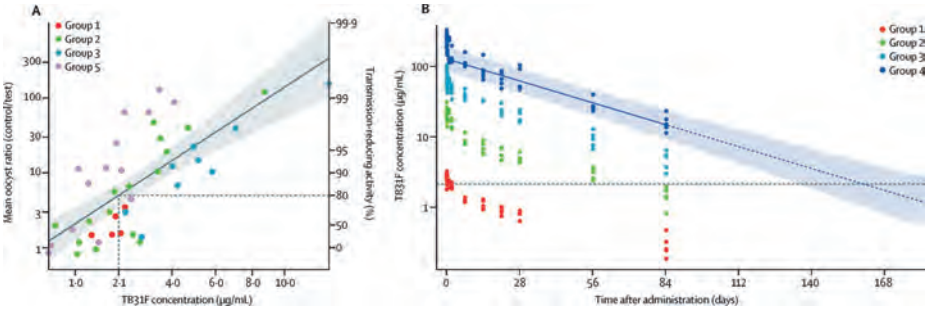

Figure 4. Estimation of IC_{80} and predicted time of more than 80% TRA. (A) TB31F concentration in serum at which 80% TRA (horizontal dashed line) is expected (IC_{80}) was calculated by linear regression to be 2·1 µg/mL (vertical dashed line). Datapoints represent measured serum TB31F concentrations and respective TRA values for individual participants in groups 1 to 5, including only those timepoints where TRA was less than 99·5%. Shaded areas represent the pointwise 95% CI for the linear association. Given that all group 4 volunteers had TRA values of more than 99·5%, no points were included in this analysis. (B) The observed TB31F concentrations of individuals (circles) were used to extrapolate TB31F concentrations over time. For each individual in group 4 (10 mg/kg), the observed concentrations in samples from day 7 to day 84 were used to extrapolate concentrations up to month 6 via linear regression. The solid line represents the geometric mean of the observed data and the dashed line represents the extrapolated linear regressions. The coloured band represents the 95% CI for the extrapolated data. The horizontal dashed black line represents the IC_{80} of 2·1 µg/mL, which is crossed on day 160 (95% CI 136–193). The confidence intervals were calculated with the geometric standard deviation assuming lognormality. TRA=transmission-reducing activity.

Discussion

Here, we did the first clinical evaluation of a *P falciparum* transmission-blocking monoclonal antibody. TB31F administration was well tolerated and reached a high TRA. All participants who received 1 mg/kg or more of TB31F intravenously displayed TRA above the 80% benchmark. At a single intravenous dose of 10 mg/kg, this functional activity was maintained throughout the 12 weeks of follow-up and was predicted to persist for 5 months. Interrupting the highly efficient transmission of *Plasmodium* parasites has the potential to reduce the global burden of malaria, and a safe and potent transmission-blocking monoclonal antibody could provide the means to achieve this.

Although this study was not powered to detect less frequent adverse events, the excellent safety profile of TB31F observed here is consistent with that of other humanised antibodies against infectious diseases.[5–7] The precautionary administration of premedication was specifically intended to dampen potential allergic and infusion reactions in this first-in-human trial; the short half-

lives of paracetamol and clemastine relative to that of TB31F are unlikely to have masked clinically relevant allergic reactions to TB31F. Notably, the most frequently observed adverse event, fatigue, is most likely a side-effect of the histamine antagonist clemastine. Larger trials are required to confirm this safety profile and to rule out less frequent side-effects and might also assess administration in absence of premedication. This modestly sized trial did allow detailed assessments of functional activity. By reaching TRA of 80% or more at a concentration of 2·1 µg/mL, TB31F is the most potent antimalarial monoclonal antibody described to date, confirming IC_{80} values of preclinical studies.[12,20]

A few limitations of our study warrant consideration. First, one participant in the subcutaneous group appeared to exhibit no or very low concentrations of TB31F in serum and a corresponding absence of TRA. A clear explanation was not identified, but human error in study product preparation or administration cannot be fully ruled out. More generally, bioavailability after subcutaneous monoclonal antibody administration might be affected by biophysical properties of the monoclonal antibody interacting with characteristics of the subcutaneous tissue at the chosen injection site.[21] Future clinical trials should continue to evaluate whether this is a tangible issue for subcutaneous administration of TB31F.

Second, our findings in healthy malaria-naive adult participants require confirmation in populations that might be a future target population for TB31F administration—eg, children in malaria-endemic settings who constitute the largest source of malaria transmission.[22] Pharmacokinetics of monoclonal antibodies in children of different age groups might vary from those in adults and from each other.[23] Additionally, protein malnutrition is more common in many resource-poor malaria-endemic regions and might affect monoclonal antibody pharmacokinetics, as observed in their use in patients with cancer.[24,25]

TB31F targets a highly conserved epitope on Pfs48/45 that contains only three very rare polymorphisms.[12] 85RF45.1, the precursor of TB31F, retains low nanomolar affinity for the recombinant protein containing these single nucleotide polymorphisms.[12] We previously showed that 85RF45.1 has cross-strain functional activity in endemic settings.[20] A post-hoc SMFA showed that sera with TB31F retained strong TRA against the genetically distant Asian isolate NF135. For these reasons, we predict that the efficacy of TB31F will not be affected by genetic variation in circulating field strains. This also makes TB31F a valuable tool to compare prevailing functional assays for

transmission-blocking vaccines by determining the association between TB31F efficacy estimates in the in-vitro SMFA and ex-vivo mosquito membrane feedings and direct skin feeding assays, in naturally infected gametocyte carriers who receive different doses of TB31F. As a next step, it is important to consider half-life extension strategies,[26–28] which are of particular interest to support dose-reduction, resulting in a more cost-effective intervention and lower administration volumes that facilitate subcutaneous administration for mass administration in malaria-endemic settings. Assuming an extension of TB31F half-life by two to four times, 80% TRA after a single dose of 10 mg/kg could be maintained for nearly 1 or 2 years, respectively (supplementary figure 6). Future studies might also administer TB31F to naturally infected gametocyte carriers to confirm its potency against genetically complex infections and directly examine the reductions that can be achieved in the proportion of mosquitoes that become infected.

Our findings support further development of TB31F as a promising cointervention tool to be studied in malaria-endemic populations, where it might be considered in seasonal settings, in mass administration campaigns to eliminate (drug-resistant) malaria, or in outbreak suppression. Mathematical modelling can inform future studies investigating the population-wide transmission-reducing potential of TB31F as an intervention strategy against malaria in different population groups at varying dosages. This includes standalone TB31F administration, and use in combination with interventions targeting other stages of the parasite lifecycle, including the recently reported CIS43LS monoclonal antibody for preventing *P falciparum* infections.[4] Given the heterogeneity of transmission, such models should address the effect of TB31F administration to not only different demographic groups (eg, distinct age categories), but also in regions of different transmission intensity and seasonality, to further guide the effective deployment of TB31F.

In summary, this trial provides highly promising initial data on TB31F, the first malaria transmission-blocking monoclonal antibody to be assessed in humans. Its safety, tolerability, and high efficacy at interrupting human-to-mosquito transmission mark TB31F out to be an important potential tool for malaria control and elimination, advocating for its further assessment in naturally infected gametocyte carriers.

Contributors

SCvdB, MJS, CFO, YW, S-ML, CRK, AJB, RWS, TB, MMJ, and MBBM conceived the study and developed the study protocol. SCvdB, MJS, SWvB, JR, KM, EL, RtH, YW, KM, CFO, MAA, RWS, TB, MMJ, and MBBM developed the methodology. YW, CRK, AJB, RWS, and CFO acquired funding. PP, YW, EL, S-ML, CRK, AJB, KM, MAA, RWS, RtH, CFO, TB, MMJ, and MBBM provided oversight and supervision. MJS, KT, KM, MAA, and MMJ did the pharmacokinetic assays. SWvB, JR, and RtH did the pharmacokinetic analysis. MvdV-B, G-JvG, and KM did the standard membrane feeding assay. SWvB and JR did the statistical analysis. SCvdB, MJS, PP, TB, MMJ, and MBBM coordinated the trial and oversaw data collection and management. SCvdB, MJS, SWvB, JR, KT, MvdV-B, G-JvG, PP, TB, KZ, MMJ, and MBBM accessed and verified the data. SCvdB, MJS, SWvB, JR, MK, RtH, JA, TB, MMJ, and MBBM analysed the data. SCvdB, MJS, SWvB, TB, MMJ, and MBBM wrote the original draft of the manuscript. SCvdB, MJS, YW, MMJ, and MBBM accessed and verified all the data. All authors had access to the raw data, reviewed and approved the final manuscript, and agreed to the submission for publication.

Data sharing

The study protocol and statistical analysis plan can be accessed via ClinicalTrials.gov, NCT04238689. Deidentified participant data that underlie the results reported in this Article will be made available upon request. Proposals should be directed to the corresponding author. Proposals will be reviewed and approved by the sponsor, investigators, and collaborators on the basis of scientific merit. After approval of a proposal, data requestors will need to sign a data access agreement. Data can be requested indefinitely.

Declaration of interests

We declare no competing interests.

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References

1. WHO. World malaria report 2021. World Health Organization, Geneva, 2021.
2. Uwimana A, Legrand E, Stokes BH, et al. Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. Nat Med 2020; 26: 1602–8.
3. Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature 2015; 526: 207–11.
4. Gaudinski MR, Berkowitz NM, Idris AH, et al. A monoclonal antibody for malaria prevention. N Engl J Med 2021; 385: 803–14.
5. The IMpact-RSV Study Group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics 1998; 102: 531–7.
6. Emu B, Fessel J, Schrader S, et al. Phase 3 study of ibalizumab for multidrug-resistant HIV-1. N Engl J Med 2018; 379: 645–54.
7. Deng R, Wang Y, Maia M, et al. Pharmacokinetics and exposure-response analysis of RG7667, a combination of two anticytomegalovirus monoclonal antibodies, in a phase 2a randomized trial to prevent cytomegalovirus infection in high-risk kidney transplant recipients. Antimicrob Agents Chemother 2018; 62: e01108–17.
8. Johnston GL, Smith DL, Fidock DA. Malaria's missing number: calculating the human component of R0 by a within-host mechanistic model of Plasmodium falciparum infection and transmission. PLOS Comput Biol 2013; 9: e1003025.
9. de Jong RM, Tebeje SK, Meerstein-Kessel L, et al. Immunity against sexual stage Plasmodium falciparum and Plasmodium vivax parasites. Immunol Rev 2020; 293: 190–215.
10. Healy SA, Anderson C, Swihart BJ, et al. Pfs230 yields higher malaria transmission-blocking vaccine activity than Pfs25 in humans but not mice. J Clin Invest 2021; 131: 146221.
11. Roeffen W, Teelen K, van As J, et al. Plasmodium falciparum: production and characterization of rat monoclonal antibodies specific for the sexual-stage Pfs48/45 antigen. Exp Parasitol 2001; 97: 45–9.
12. Kundu P, Semesi A, Jore MM, et al. Structural delineation of potent transmission-blocking epitope I on malaria antigen Pfs48/45. Nat Commun 2018; 9: 4458.
13. Lennartz F, Brod F, Dabbs R, et al. Structural basis for recognition of the malaria vaccine candidate Pfs48/45 by a transmission blocking antibody. Nat Commun 2018; 9: 3822.
14. Singh SK, Plieskatt J, Chourasia BK, et al. A reproducible and scalable process for manufacturing a Pfs48/45 based Plasmodium falciparum transmission-blocking vaccine. Front Immunol 2021; 11: 606266.
15. van der Kolk M, De Vlas SJ, Saul A, et al. Evaluation of the standard membrane feeding assay (SMFA) for the determination of malaria transmission-reducing activity using empirical data. Parasitology 2005; 130: 13–22.
16. Walk J, Reuling IJ, Behet MC, et al. Modest heterologous protection after Plasmodium falciparum sporozoite immunization: a double-blind randomized controlled clinical trial. BMC Med 2017; 15: 168.
17. Miura K, Deng B, Tullo G, et al. Qualification of standard membrane-feeding assay with Plasmodium falciparum malaria and potential improvements for future assays. PLoS One 2013; 8: e57909.

18. Sauerwein RW, Bousema T. Transmission blocking malaria vaccines: assays and candidates in clinical development. Vaccine 2015; 33: 7476–82.
19. Churcher TS, Blagborough AM, Delves M, et al. Measuring the blockade of malaria transmission–an analysis of the Standard Membrane Feeding Assay. Int J Parasitol 2012; 42: 1037–44.
20. de Jong RM, Meerstein-Kessel L, Da DF, et al. Monoclonal antibodies block transmission of genetically diverse Plasmodium falciparum strains to mosquitoes. NPJ Vaccines 2021; 6: 101.
21. Turner MR, Balu-Iyer SV. Challenges and opportunities for the subcutaneous delivery of therapeutic proteins. J Pharm Sci 2018; 107: 1247–60.
22. Andolina C, Rek JC, Briggs J, et al. Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. Lancet Infect Dis 2021; 21: 1568–78.
23. Malik P, Edginton A. Pediatric physiology in relation to the pharmacokinetics of monoclonal antibodies. Expert Opin Drug Metab Toxicol 2018; 14: 585–99.
24. Smerdon C, Kim H. Association of malnutrition measures with the risk of malaria in Sierra Leone. Curr Dev Nutr 2020; 4: 908.
25. Trobec K, Kerec Kos M, von Haehling S, et al. Pharmacokinetics of drugs in cachectic patients: a systematic review. PloS One 2013; 8: e79603.
26. Robbie GJ, Criste R, Dall'acqua WF, et al. A novel investigational Fc-modified humanized monoclonal antibody, motavizumab-YTE, has an extended half-life in healthy adults. Antimicrob Agents Chemother 2013; 57: 6147–53.
27. Gaudinski MR, Coates EE, Houser KV, et al. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: a phase 1 open-label clinical trial in healthy adults. PLoS Med 2018; 15: e1002493.
28. Yu XQ, Robbie GJ, Wu Y, et al. Safety, tolerability, and pharmacokinetics of MEDI4893, an investigational, extended-half-life, anti-Staphylococcus aureus alpha-toxin human monoclonal antibody, in healthy adults. Antimicrob Agents Chemother 2016; 61: e01020–16.

Supplementary Material

Please scan the QR code below to access the supplementary material for Chapter 6:

CHAPTER 7

Monoclonals against malaria: the promise of passive protection

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COMMENT

In *The Lancet Infectious Diseases*, Kirsten Lyke and colleagues[1] report the findings of the third part of a three-part trial evaluating low doses of the monoclonal antibody CIS43LS, administered intravenously or subcutaneously, for protection against *Plasmodium falciparum* in controlled human malaria infection.

WHO estimated that global malaria deaths rose to 627 000 in 2020. Novel interventions are needed to reduce the malaria burden, caused mostly by *P falciparum*. Progress with *P falciparum* vaccines has been encouraging, including WHO's recommendation to roll out RTS,S, although the efficacy and durability of current vaccines in infants and children remains suboptimal. Achieving sufficient protection through vaccination might be hindered by multiple factors, including the specificity of T-cell and B-cell responses against the vaccine (ie, the cryptic nature of protective epitopes), functional immaturity of young children's immune systems, and immunotolerance induced by previous parasite exposure.[2] As shown more than half a century ago in seminal studies using human immune serum, passive immunisation (albeit now through administration of rationally selected, highly active monoclonal antibodies) represents an effective means to bypass such obstacles.[3]

The human IgG1 monoclonal antibody CIS43LS prevents malaria by neutralising sporozoites, the parasite forms that are inoculated during an infectious mosquito bite. CIS43LS inhibits sporozoites from invading hepatocytes, the first stage of the parasite lifecycle, so that both the pathogenic blood stage and onward parasite transmission are averted. Using an approach pioneered in HIV research, the monoclonal antibody CIS43 was originally derived directly from a human participant who received immunisation with radiation-attenuated *P falciparum* sporozoites (PfSPZ vaccine). Uniquely, it targets a highly conserved junctional epitope on the prominent circumsporozoite protein of *P falciparum*.[4] Modifications were made within the fragment crystallisable region of CIS43, extending its half-life and resulting in CIS43LS, which has an estimated half-life of 80 days.

The study team behind CIS43LS previously showed that participants with no previous malaria infections who received a high intravenous dose (20 mg/kg or 40 mg/kg) of CIS43LS were sterilely protected against controlled human malaria infection.[5] In part C of the VRC 612 trial, Lyke and colleagues[1] describe the safety, tolerability, pharmacokinetics, and protective efficacy of low-dose

intravenous and subcutaneous administration of CIS43LS. 31 participants were enrolled (one subsequently withdrew and was replaced) and assigned to receive doses of 1 mg/kg (n=7), 5 mg/kg (n=4), and 10 mg/kg (n=3) intravenously and 5 mg/kg (n=4) and 10 mg/kg (n=4) subcutaneously, or to the control group (n=8). CIS43LS administration was safe and well tolerated by either route at all doses, causing no severe or serious adverse events. All control participants (who received no CIS43LS) developed parasitaemia following controlled human malaria infection by the bites of five *P falciparum*-infected mosquitoes, whereas all participants who received 5 mg/kg or 10 mg/kg CIS43LS, plus three of seven participants who received 1 mg/kg, were sterilely protected against parasitaemia.

In combination with pharmacokinetic measurements, the minimum serum CIS43LS concentration required at the time of infection for 90% or greater protection was estimated to be 22·5 µg/mL (95% CI 12·1–41·7). Such estimates are crucial to the further clinical development and implementation of monoclonal antibodies, as they dictate the dose and frequency of administration required to protect recipients over a given timeframe. However, exposure to five infectious mosquitoes simultaneously probably represents a more stringent challenge than occurs in almost all endemic settings, and genetically diverse *P falciparum* strains might be differentially susceptible to CIS43LS. The protective threshold should therefore be reassessed in the context of an ongoing trial of CIS43LS (NCT04329104).

The junctional epitope primarily targeted by CIS43LS appears to be highly conserved among *P falciparum* isolates, and mutational rates in this eukaryote are lower than in viruses. Nevertheless, such a narrow protective target evidently comes under great selective pressure and *P falciparum* has in the past repeatedly shown the evolutionary ability to escape selective pressure, for example from chemotherapy. CIS43LS's long half-life could even represent a vulnerability, resulting in prolonged subinhibitory serum concentrations that help select for isolates with lower CIS43LS binding affinity. It will thus be crucial to monitor breakthrough infections in field trials. One potential strategy to decrease the spread of CIS43LS-resistant *P falciparum* strains could be the coadministration of a transmission-blocking monoclonal antibody, such as TB31F, to prevent the uptake and dispersal of CIS43LS-escape mutants by mosquitoes.[6]

The finding that subcutaneous administration of CIS43LS is both well tolerated and efficacious[1] holds substantial promise for large-scale roll-out in low-resource settings, particularly in young children, as it is quicker and requires fewer resources and less-skilled staff than intravenous administration. Recipients would still require repeated doses to maintain passive protection throughout childhood, even given the extended half-life of CIS43LS. Moreover, the passive protection afforded by CIS43LS might hinder the development of naturally acquired immunity, resulting in a rebound effect after administration is discontinued. In this context, it is encouraging that coadministration of CIS43LS and the subunit *P falciparum* vaccine candidate R21 has been shown to result in synergistic protection, at least in a mouse model,[7] and this should be assessed in humans. Other potential strategies proposed to further develop CIS43LS include site-saturation mutagenesis[8] to increase its potency and expression in a viral vector[9] to increase its longevity. Meanwhile, even more potent monoclonal antibodies are entering clinical development.[10]

Ultimately, policy decisions regarding the implementation of monoclonal antibodies against malaria, such as CIS43LS, must be based on their relative long-term efficacy and cost-effectiveness in low-resource settings. In this regard, monoclonal antibodies will need to be offset against not only active vaccination strategies and other vertically designed interventions against malaria, but also investments in horizontal (public) health-care provision that affect the full spectrum of morbidity and mortality in communities.

Competing Interests

We declare no competing interests.

References

1. Lyke KE, Berry AA, Mason K, et al. Low-dose intravenous and subcutaneous CIS43LS monoclonal antibody for protection against malaria (VRC 612 Part C): a phase 1, adaptive trial. Lancet Infect Dis 2023; published online Jan 25. https://doi.org/10.1016/S1473-3099(22)00793-9.
2. Montes de Oca M, Good MF, McCarthy JS, et al. The impact of established immunoregulatory networks on vaccine efficacy and the development of immunity to malaria. J Immunol 2016; 197: 4518–26.
3. Aleshnick M, Florez-Cuadros M, Martinson T, et al. Monoclonal antibodies for malaria prevention. Mol Ther 2022; 30: 1810–21.
4. Kisalu NK, Idris AH, Weidle C, et al. A human monoclonal antibody prevents malaria infection by targeting a new site of vulnerability on the parasite. Nat Med 2018; 24: 408–16.
5. Gaudinski MR, Berkowitz NM, Idris AH, et al. A monoclonal antibody for malaria prevention. N Engl J Med 2021; 385: 803–14.
6. van der Boor SC, Smit MJ, van Beek SW, et al. Safety, tolerability, and Plasmodium falciparum transmission-reducing activity of monoclonal antibody TB31F: a single-centre, open-label, first-in-human, dose-escalation, phase 1 trial in healthy malaria-naive adults. Lancet Infect Dis 2022; 22: 1596–605.
7. Wang LT, Pereira LS, Kiyuka PK, et al. Protective effects of combining monoclonal antibodies and vaccines against the Plasmodium falciparum circumsporozoite protein. PLoS Pathog 2021; 17: e1010133.
8. Banach BB, Tripathi P, Da Silva Pereira L, et al. Highly protective antimalarial antibodies via precision library generation and yeast display screening. J Exp Med 2022; 219: e20220323.
9. Kisalu NK, Pereira LD, Ernste K, et al. Enhancing durability of CIS43 monoclonal antibody by Fc mutation or AAV delivery for malaria prevention. JCI Insight 2021; 6: e143958.
10. Wu RL, Idris AH, Berkowitz NM, et al. Low-dose subcutaneous or intravenous monoclonal antibody to prevent malaria. N Engl J Med 2022; 387: 397–407.

Part 3: Beyond malaria: expanding vaccine research

CHAPTER 8

First-in-human use of a modular capsid virus-like vaccine platform: an open-label, non-randomised, phase 1 clinical trial of the SARS-CoV-2 vaccine ABNCoV2

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Summary

Background

Capsid virus-like particles (cVLP) have proven safe and immunogenic and can be a versatile platform to counter pandemics. We aimed to clinically test a modular cVLP COVID-19 vaccine in individuals who were naive to SARS-CoV-2.

Methods

In this phase 1, single-centre, dose-escalation, adjuvant-selection, open-label clinical trial, we recruited participants at the Radboud University Medical Center in Nijmegen, Netherlands, and sequentially assigned them to seven groups. Eligible participants were healthy, aged 18–55 years, and tested negative for SARS-CoV-2 and anti-SARS-CoV-2 antibodies. Participants were vaccinated intramuscularly on days 0 and 28 with 6 μg, 12 μg, 25 μg, 50 μg, or 70 μg of the cVLP-based COVID-19 vaccine (ABNCoV2). A subgroup received MF59-adjuvanted ABNCoV2. Follow-up was for 24 weeks after second vaccination. The primary objectives were to assess the safety and tolerability of ABNCoV2 and to identify a dose that optimises the tolerability–immunogenicity ratio 14 days after the first vaccination. The primary safety endpoint was the number of related grade 3 adverse events and serious adverse events in the intention-to-treat population. The primary immunogenicity endpoint was the concentration of ABNCoV2-specific antibodies. The trial is registered with ClinicalTrials.gov, NCT04839146.

Findings

45 participants (six to nine per group) were enrolled between March 15 and July 15, 2021. Participants had a total of 249 at least possibly related solicited adverse events (185 grade 1, 63 grade 2, and one grade 3) within a week after vaccination. Two serious adverse events occurred; one was classified as a possible adverse reaction. Antibody titres were dose-dependent with levels plateauing at a vaccination dose of 25–70 μg ABNCoV2. After second vaccination, live virus neutralisation activity against major SARS-CoV-2 variants was high but was lower with an omicron (BA.1) variant. Vaccine-specific $IFN\gamma^+$ $CD4^+$ T cells were induced.

Interpretation

Immunisation with ABNCoV2 was well tolerated, safe, and resulted in a functional immune response. The data support the need for additional clinical development of ABNCoV2 as a second-generation SARS-CoV-2

vaccine. The modular cVLP platform will accelerate vaccine development, beyond SARS-CoV-2.

Research in context

Evidence before this study

In the COVID-19 pandemic, mRNA vaccines, and then vectored SARS-CoV-2 vaccines, spearheaded market entry, whereas protein-based candidates failed in early clinical development due to low immunogenicity. Virus-like particle (VLP) vaccines are highly immunogenic, safe, and can be effective over long periods of time (eg, against human papillomavirus); however, development of VLP-based vaccines is often precluded by complex manufacturing procedures and the limited propensity of antigens to spontaneously form particles. We developed a simple modular capsid VLP platform that allows rapid development of VLP-based vaccines and we aimed for proof-of-concept with the SARS-CoV-2 vaccine ABNCoV2. ABNCoV2 generated robust vaccine dose-dependent neutralising antibody responses in preclinical studies and protected SARS-CoV-2-challenged Rhesus macaques. We report results of the first-in-human trial of ABNCoV2. We searched PubMed on Aug 4, 2022, for clinical trials testing SARS-CoV-2 virus-like particle vaccines with search terms "SARS-CoV-2 AND vaccine AND (VLP OR virus-like) AND (clinical trial [Filter])", with no restrictions on publication date or language. We found one publication that reported the interim safety and immunogenicity data of a phase 1 trial with a plant-derived virus-like particle vaccine for COVID-19.

Added value of this study

Next-generation vaccines with improved tolerability, broad and durable protection, global accessibility, and transmission-blocking activity will be required for control of the SARS-CoV-2 pandemic. Our data show that ABNCoV2 is well tolerated and elicits high antibody titres, high titres of cross-neutralisation antibodies, and robust cellular responses with the preferred T-helper-1 cell pattern indicative of a protective immune status. Beyond SARS-CoV-2, the study provides successful proof-of-concept of a modular capsid VLP platform for the development of improved vaccines for globally relevant infectious diseases and pathogens of concern.

Implications of all the available evidence
ABNCoV2 is a promising complementary SARS-CoV-2 vaccine candidate and has proceeded into phase 3 clinical development. A two-dose schedule of ABNCoV2 was well tolerated and induced rapid and durable immunity. Distribution and storage of ABNCoV2 are less demanding compared with current SARS-CoV-2 vaccines, which will ease its global supply once available on the market. Modular capsid VLPs are a platform for the development of next-generation vaccines against SARS-CoV-2 and other infectious diseases.

Introduction

As of July 9, 2022, there have been over 550 million cases of COVID-19 worldwide, with more than 6 million deaths.[1] Vaccines against SARS-CoV-2, the causative virus, have been instrumental in controlling the pandemic. Vaccination has been implemented globally at unprecedented pace to protect susceptible populations, reduce spread, safeguard health-care systems, and diminish the global social and economic impact of non-pharmaceutical interventions to reduce COVID-19 transmission.[2] However, reduced vaccine effectiveness against new SARS-CoV-2 variants, ongoing trans_mission, and the absence of universal access are major challenges. Heterologous vaccination with different existing COVID-19 vaccines is an approach to broaden protection; however, so far, it has provided little benefit over homologous boosters.[3] Second-generation COVID-19 vaccines should ideally induce durable cross-protective and transmission-blocking immune responses, while being compatible with globally equitable use.

We developed a novel modular vaccine platform based on capsid virus-like particles (cVLP) that are used as scaffolds for antigen display.[4] This cVLP platform uses a split-protein Tag–Catcher conjugation system (similar to the SpyTag–SpyCatcher technology)[5] to allow for directional, high-density, covalent attachment of protein antigens on the cVLP surface. This cVLP platform was used to develop a COVID-19 vaccine, ABNCoV2, by attaching the receptor binding domain (RBD) of the SARS-CoV-2 Spike glycoprotein.[4] The increased avidity and particle size can promote uptake by antigen presenting cells, lymph node trafficking, and B-cell activation.[6] In preclinical studies in mice, ABNCoV2 was immunogenic and induced high titres of neutralising antibodies.[4] cVLP-based vaccines have been successfully marketed and have

been shown to be highly effective over long periods of time (eg, against human papillomavirus).[7] cVLP-based vaccines can be safely used in people who are immunocompromised and in older people, two populations at high risk of severe COVID-19.[8,9]

Here, we report the results of the first-in-human clinical trial COUGH-1, designed to assess the safety, tolerability, and immunogenicity of ABNCoV2 in participants who were naive to SARS-CoV-2.

Methods

Study design and participants

COUGH-1 was a phase 1, single centre, sequential dose-escalation, adjuvant-selection, open-label trial, conducted at the Radboud University Medical Center in Nijmegen, Netherlands. Healthy participants aged 18–55 years, with no history of SARS-CoV-2 infection or vaccination were recruited. Following written informed consent, all participants underwent physical examination, haematological and biochemical screening, and were tested for current or past infection with SARS-CoV-2, HIV, and hepatitis B and hepatitis C viruses. Full details of the inclusion and exclusion criteria are provided in the protocol.

Ethical approval was granted by the Central Committee on Research Involving Human Subjects (NL76192.000.20). The trial is registered with ClinicalTrials.gov (NCT04839146) and the Netherlands Trial Register (NL9334).

Procedures

ABNCoV2 was administered by two intramuscular injections of 0·5 mL, 28 days apart, in the deltoid muscle of the non-dominant arm and, subsequently, the other arm. At each dose escalation, one participant was inoculated, followed by the rest of the group one week later, together with the first participant of the next group. Follow-up visits were done on days 1, 2, 7, and 14 after each vaccination and on days 42, 91, and 168 after the second vaccination. Adverse events were captured during on-site visits, through structured diaries, and by daily monitoring of body temperature for 1 week after each vaccination. Local and systemic adverse events were solicited until 7 days after ABNCoV2 administration. Unsolicited adverse events and serious adverse events (SAEs) were recorded until the end of study.

Allocation to dosage and combination with MF59-adjuvant was by sequence of enrolment. The predefined escalation schedule started with 6 µg (groups 1A and 1B), followed by 12 µg (groups 2A and 2B), 25 µg (groups 3A, 3B, and 6), 50 µg (groups 4 and 7), and 70 µg (group 5) ABNCoV2. Dose escalation occurred in groups of six participants, starting with split groups for the first three lowest doses, in which half (n=3) of the participants received the non-adjuvanted vaccine (groups 1A, 2A, and 3A) and half received the MF59-adjuvanted vaccine (groups 1B, 2B, and 3B). Additional dose escalation and the decision of whether to use adjuvant in group 4 and above depended on a review of the accumulated data up to 14 days after first vaccination in group 3B by an independent Safety Monitoring Committee (SMC). At completion of the dose escalation, the two doses nearest the optimal tolerability–immunogenicity ratio continued enrolment (into groups 6 and 7) until 12 participants received these doses of ABNCoV2.

Participants were allowed to enrol into the Dutch SARS-CoV-2 vaccination programme on the condition that it was later than 4 weeks after the final scheduled ABNCoV2 vaccination. In those enrolled in the national programme, additional follow-up visits before and two weeks after the additional vaccination were done.

ABNCoV2 consists of the *Acinetobacter* Phage 205 (AP205) cVLP produced in *Escherichia coli* and the Wuhan SARS-CoV-2 Spike RBD antigen aa319–591 (RBD; QIA0044.1), expressed in S2 cells (supplementary figure 1). Upon mixing the two components, a covalent isopeptide bond forms between the split-protein Tag and Catcher, which are genetically fused to the cVLP and antigen sequence, respectively.[4] The final purified RBD-cVLP contains roughly 72 RBD antigens per particle. ABNCoV2 was stored frozen at −20°C (±5°C) and reconstituted in phosphate buffered saline with and without MF59. Formulated vaccines were stored at 2–8°C and used within 24 h. MF59 is an oil-in-water emulsion containing squalene, polysorbate 80, and sorbitan trioleate and is marketed as part of the influenza vaccine, Fluad Tetra (Seqirus, Holly Springs, NC, USA). MF59 mainly acts through enhanced recruitment of immune cells to the injection site and has immune-stimulatory effects in T-helper cell deficient conditions.[10] The MF59 adjuvant was manufactured and provided by Seqirus.

Outcomes

The primary safety endpoint of this trial was the number of at least possibly related grade 3 adverse events and SAEs from time of first ABNCoV2

administration to the end of the follow-up period. The secondary safety endpoint was the number and severity of solicited adverse events within 1 week following administration of ABNCoV2. Solicited local adverse events were defined as pain, tenderness, erythema, and induration at the injection site. Solicited systemic adverse events were defined as headache, fatigue, fever, drowsiness, and chills. Causality to the study interventions was graded by the investigators (MJS, MBPAA, MBBM, and BGM) as unrelated, unlikely related, possibly, probably, or definitely related. Severity of adverse events was graded as mild (grade 1), moderate (grade 2), or severe (grade 3). Verbatim-recorded adverse events were coded using the Medical Dictionary for Regulatory Activities (version 24.1). For solicited and laboratory adverse events, the US Food and Drug Administration toxicity grading scale was used (supplementary table 1 and 2).

The primary immunogenicity endpoint was the concentration of vaccine-specific IgG antibodies 14 days after first vaccination. Exploratory immunogenicity endpoints included the concentration of vaccine-specific antibodies at baseline, during immunisation, and at follow-up. RBD-specific total IgG titres were measured by ELISA, as previously described (supplementary methods).[11] RBD-specific $CD4^+$ and $CD8^+$ T cells were measured by flow cytometry following peptide stimulation (supplementary methods).

Another exploratory endpoint was virus neutralisation of the ancestral isolate FR-4286 (B.1) and variants of concern: alpha (B.1.1.7), beta (B.1.351), and delta (B.1.617.2). We assessed serum from baseline and during immunisation and follow-up in a 50% plaque reduction neutralisation test ($PRNT_{50}$). The incidence of infection with omicron variants increased sharply after the completion of the trial. Therefore, measurement of omicron (BA.1) virus neutralisation was amended to the assay list and compared with an ancestral (D614G) variant and delta variants. These measurements were done independently of the originally planned virus neutralisation panel. Virus neutralisation assays were done as previously described (supplementary methods).[4,12]

Statistical analysis

This study was an exploratory phase 1 clinical trial. The sample size was chosen to allow detection of large differences in adverse events and RBD-specific antibodies. The study was powered to detect at least one common (≥5%) adverse reaction with 90% power and, until the first SMC review, a

ten-fold difference in antibody titre between non-adjuvanted and adjuvanted ABNCoV2 with 80% power. The immunogenicity assumptions were derived from preceding non-human primate studies.[13]

All analyses were programmed using R (version 4.1.2), and data wrangling and figures were produced with the package tidyverse (version 1.3.1).

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Screening took place between March 11, 2021, and June 14, 2021. In total, 45 eligible individuals who were naive to SARS-CoV-2 were enrolled and allocated to one of seven groups (six to nine per group; figure 1). Baseline demographics of the participants were similar among groups (table 1). Vaccinations were given between March 15, 2021, and July 15, 2021. The second ABNCoV2 vaccination was given outside the prespecified time window (27–29 days) for two (4%) participants (day 30 and 35) for logistical reasons. 44 (98%) of 45 participants completed all the follow-ups. One (2%) participant in group 3A did not attend the final follow-up visit in person due to emigration but was included in the analysis. An additional unscheduled visit was conducted after the occurrence of a suspected unexpected serious adverse reaction (SUSAR). The final study visit was on Feb 25, 2022.

Every participant had at least one adverse event. In total, 651 adverse event episodes occurred (465 grade 1, 175 grade 2, nine grade 3, two SAE). Of those adverse events, 249 (38%) were solicited (185 grade 1, 63 grade 2, and one grade 3). Overall, ABNCoV2 was well tolerated (figure 2A and figure 2B). One unrelated SAE occurred 8 days after the second vaccination in a participant from group 7 (traumatic ligament rupture requiring hospitalisation). A second SAE was a superficial basal cell carcinoma on each upper arm in a participant from group 1B. The superficial basal cell carcinoma was diagnosed approximately 16 weeks after the second vaccination. The participant had Fitzpatrick skin type 1[14] and a medical history of basal cell carcinoma and melanoma. Based on the temporal and anatomical relationship between basal cell carcinoma and vaccination, the adverse event was classified as a SUSAR. It was successfully treated by radical

excision. No further lesions were detected in any of the other participants during the study, including in an extra visit after the last planned follow-up.

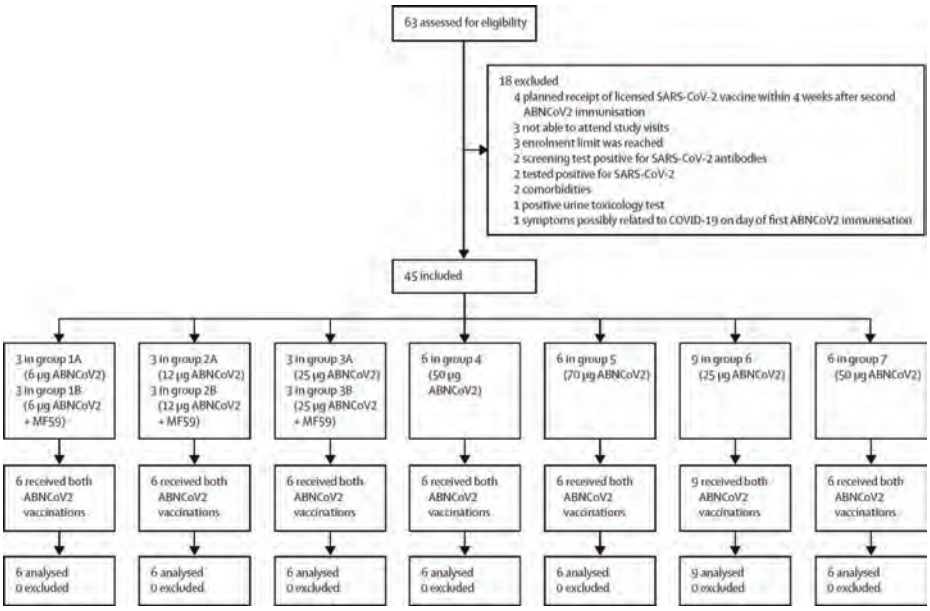

Figure 1. Trial profile.

	Median age, years (range)	Median bodyweight, kg (range)	BMI, kg/m^2 (range)	Sex, female:male ratio (%)
Group 1A (n=3)	31·0 (21·0–35·0)	79·0 (61·0–96·0)	23·0 (22·1–31·0)	2:1 (67%)
Group 1B (n=3)	27·0 (22·0–52·0)	66·0 (59·0–69·6)	22·4 (18·4–24·4)	3:0 (100%)
Group 2A (n=3)	25·0 (23·0–34·0)	78·0 (76·0–89·4)	26·0 (24·9–32·1)	2:1 (67%)
Group 2B (n=3)	37·0 (22·0–37·0)	88·0 (62·0–94·0)	24·9 (22·8–28·4)	1:2 (33%)
Group 3A (n=3)	27·0 (22·0–28·0)	70·1 (61·2–79·0)	23·6 (20·5–25·4)	2:1 (67%)
Group 3B (n=3)	48·0 (33·0–54·0)	81·0 (72·8–91·0)	26·4 (24·9–26·9)	2:1 (67%)
Group 4 (n=6)	25·5 (20·0–44·0)	75·0 (60·0–97·0)	25·0 (17·5–27·3)	4:2 (67%)
Group 5 (n=6)	20·5 (20·0–46·0)	77·5 (66·0–90·0)	23·7 (20·5–26·6)	3:3 (50%)
Group 6 (n=9)	24·0 (21·0–45·0)	76·6 (61·0–91·0)	23·3 (20·4–29·9)	4:5 (44%)
Group 7 (n=6)	24·0 (18·0–29·0)	69·5 (60·0–88·0)	24·5 (20·5–27·1)	3:3 (50%)
Overall (n=45)	26·0 (18·0–54·0)	76·0 (59·0–97·0)	24·2 (17·5–32·1)	26:19 (58%)

Table 1. Baseline demographics of the study participants. Doses of study groups were 6 µg ABNCoV2 in group 1A, 6 µg ABNCoV2+MF59 in group 1B, 12 µg ABNCoV2 in group 2A, 12 µg ABNCoV2+MF59 in group 2B, 25 µg ABNCoV2 in group 3A, 25 µg ABNCoV2+MF59 in group 3B, 50 µg ABNCoV2 in group 4, 70 µg ABNCoV2 in group 5, 25 µg ABNCoV2 in group 6, and 50 µg ABNCoV2 in group 7.

Two laboratory abnormalities occurred. One participant had grade 1 eosinophilia on day 7 after the second administration of 25 µg ABNCoV2. Another participant had dacrocytes (teardrop-shaped erythrocytes) in the whole blood cell count on day 7 after second administration of 50 µg ABNCoV2. Other haematological parameters were normal in these two patients. Both laboratory abnormalities were not associated with symptoms and normalised at the next follow-up visit (7 days later).

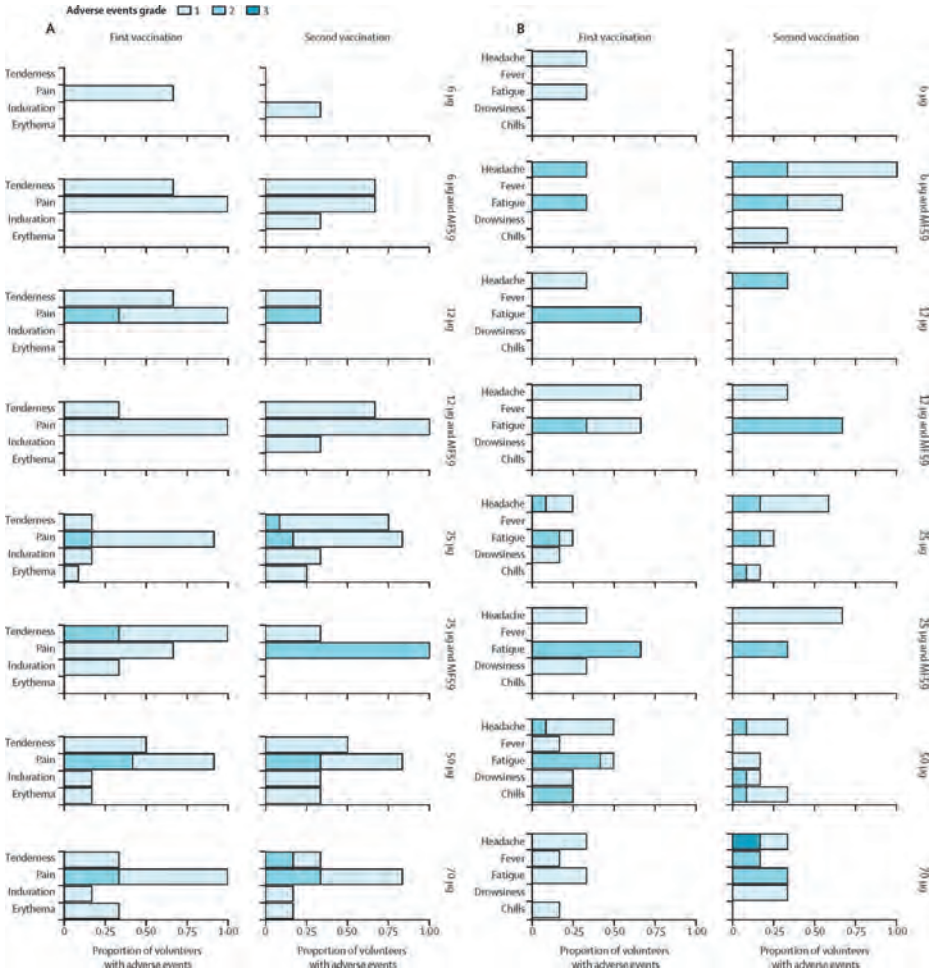

Figure 2. Local and systemic reactions following ABNCoV2 vaccination. (A) Related solicited local adverse events. (B) Related solicited systemic adverse events. Data represent the proportion of participants who had an adverse event of the indicated severity. The highest severity grade is shown in case there were multiple episodes of a given adverse event per participant. ABNCoV2 dose and vaccine formulation (with or without MF59) are indicated on the right side.

ABNCoV2 induced seroconversion after the second vaccination in all participants irrespective of dose amount and adjuvant. The decision as to whether to proceed with or without adjuvant in groups 4 to 7 was based on the concentration of RBD-specific antibodies of group 1 to group 3 (6 µg, 12 µg, and 25 µg) up to day 14 after the second vaccination (figure 3A). RBD-specific antibody IgG titres 14 days after the second vaccination were dose-dependent with a plateauing response pattern at around 25 µg (figure 3B). On the basis of these results, the decision was made to retest 25 µg and 50 µg as the optimal doses in group 6 and group 7 to increase the power to detect adverse events. In total, 12 participants received the optimal doses of 25 µg and 50 µg ABNCoV2. The concentration of RBD-specific antibodies of the groups receiving the optimal doses up to day 42 after the first vaccination is shown by locally estimated scatterplot smoothing fit local regression (figure 3C). The concentration of RBD-specific antibodies decayed gradually during the follow-up and could be boosted with a licensed SARS-CoV-2 vaccine in participants receiving the optimal doses (figure 3D, supplementary figure 3). RBD-specific $CD4^+$ T cells were induced following immunisation with two doses of ABNCoV2 (figure 4A). The phenotype of responding $CD4^+$ T cells was mainly IFNγ positive, with most of the cells coexpressing TNF and CD137 (supplementary figure 2). Some cells also expressed the degranulation marker CD107a. The 50 µg vaccine dose induced a higher $CD4^+$ T-cell response than 25 µg ABNCoV2, but this did not extend to the 70 µg dosage. SARS-CoV-2 RBD-specific $CD8^+$ T cells were only marginally increased (figure 4B). Robust in-vitro activity was observed in live virus neutralisation assays 14 days after the second vaccination. $PRNT_{50}$ titres were induced by all the different ABNCoV2 doses tested with and without adjuvant MF59 against an early B.1 isolate, FR-4286, representing ancestral variants. The WHO 20/136 standard (supplementary table 4) lies in the same range as the neutralising antibody titres after vaccination with adjuvanted ABNCoV2 and at doses higher than 25 µg (figure 4C). Post-hoc analysis showed six-fold (95% CI 3–11) higher levels of in-vitro neutralisation activity in adjuvanted than in non-adjuvanted vaccinees. Furthermore, strong cross neutralisation was seen using serum samples from the 25 µg ABNCoV2 dose groups with and without MF59 adjuvant for the early B.1 isolate (FR-4286) and for variants of concern B.1.1.7 (alpha), B.1.351 (beta), and B.1.617.2 (delta; figure 4D). There was no reduction in neutralisation capacity against alpha or delta. A 2·2-fold reduction was seen against the beta variant virus. An independent neutralisation assay showed a 66-fold decrease in activity when comparing the omicron BA.1 with an ancestral variant (D614G; figure 4E). The same trend towards higher neutralisation titres in vaccinees receiving adjuvanted ABNCoV2 was present.

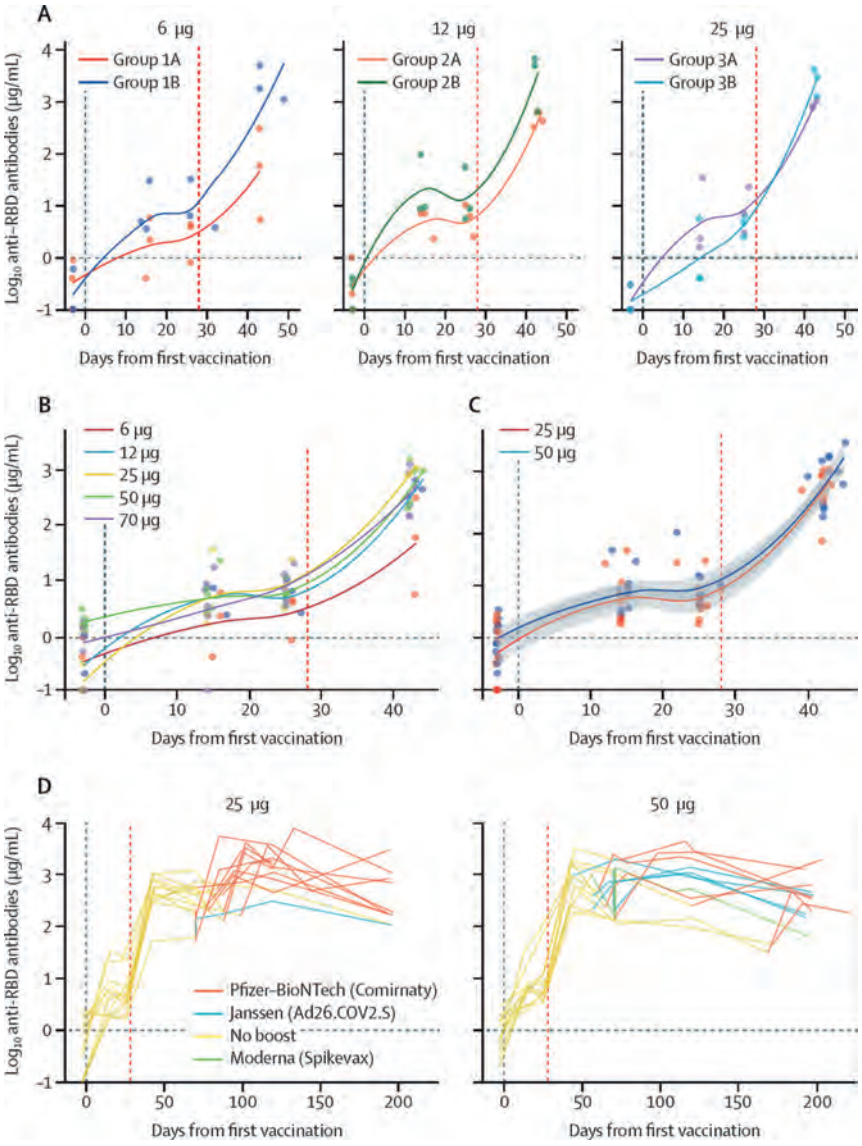

Figure 3. SARS-CoV-2 RBD-specific antibodies. Vertical lines indicate the first and second ABNCoV2 vaccination (28 days after first vaccination). (A) Concentration of RBD-specific antibodies of groups 1 to 3 (6 µg, 12 µg, and 25 µg ABNCoV2; non-adjuvanted [groups labelled A] and MF59-adjuvanted [groups labelled B]) up to day 42 after the first vaccination (14 days after the second vaccination). (B) Concentration of RBD-specific antibodies of groups 1A, 2A, 3A, 4, and 5 (6 µg, 12 µg, 25 µg, 50 µg, and 70 µg) 14 days after second vaccination. (C) Concentration of RBD-specific antibodies of groups receiving the optimal doses 25 µg and 50 µg ABNCoV2 until day 42 after the first vaccination. (D) Concentration of RBD-specific antibodies of groups receiving the optimal doses 25 µg and 50 µg ABNCoV2 until day 196 after the first vaccination (end-of-study visit). Different colours indicate types of licensed SARS-CoV-2 vaccines that participants received during the follow-up period. RBD=receptor binding domain.

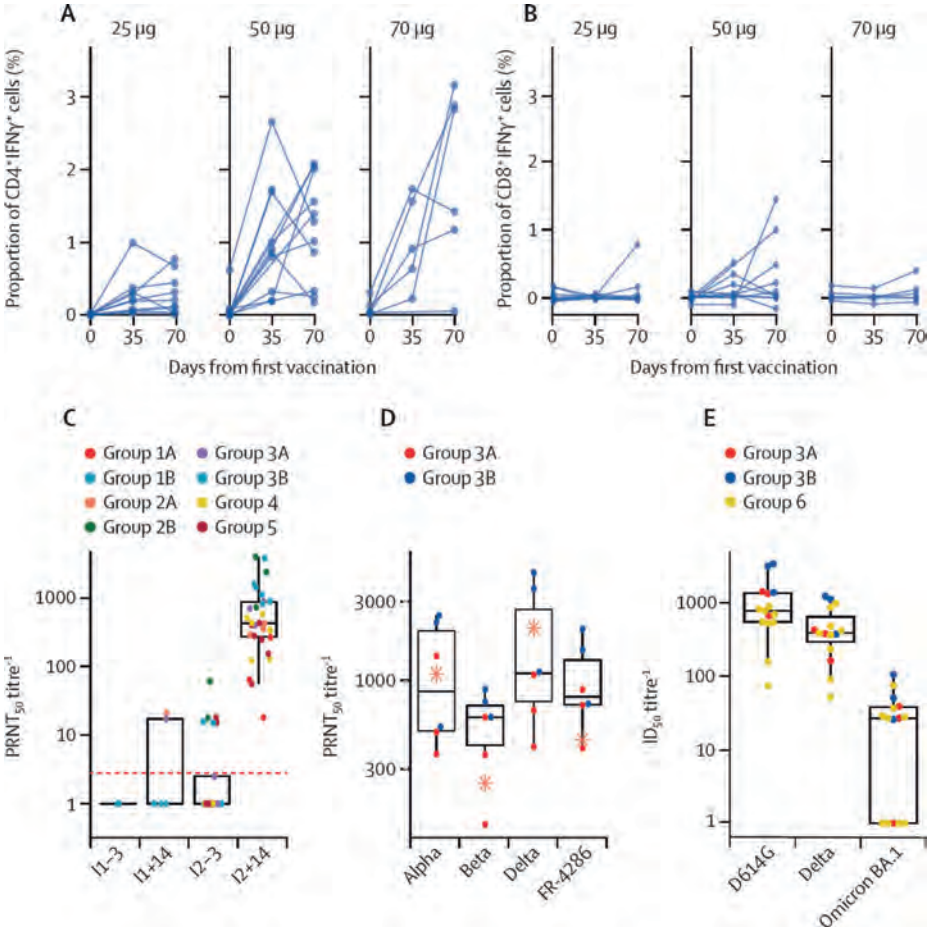

Figure 4. Cellular and functional immune response against SARS-CoV-2. IFNγ+ cells after stimulation with SARS-CoV-2 Spike RBD class 1 and class 2 restricted pooled peptides on CD4+ T cells (A) and CD8+ T cells (B). Values are corrected for activation (no peptide). (C) SARS-CoV-2 neutralisation responses. The red line indicates the WHO 20/136 standard. (D) Virus neutralisation of alpha (B.1.1.7), beta (B.1.351), delta (B.1.617.2), and FR-4286 (B.1). Red stars indicate $PRNT_{50}$ values of the WHO standard 20/136. (E) Neutralisation titres (ID_{50}) from vaccination groups 3A (n=3), 3B (n=3), and 6 (n=9) against the ancestral D614G, delta, and omicron SARS-CoV-2 variants. I1−3=3 days before the first vaccination. I1+14=14 days after first vaccination. I2−3=3 days before the second vaccination. I2+14=14 days after second vaccination. RBD=receptor binding domain. ID_{50}=50% inhibitory dilution. $PRNT_{50}$=50% plaque reduction neutralisation test.

Discussion

In this first-in-human clinical trial of ABNCoV2, we tested a modular cVLP platform that combines flexibility in the selection of antigens with improved immunogenicity as well as good tolerability and safety in preclinical models. We found that ABNCoV2 was well tolerated and induced high IgG antibody responses against the SARS-CoV-2 RBD, peaking at 2 weeks after the second vaccination. Serum samples showed functional activity against major SARS-CoV-2 variants, antibody concentrations remained high over several months, and cellular responses were robust, with a T-helper-1 cell pattern indicative of a protective immune status.[15] The immune response to the vaccine antigen was dose-dependent and MF59 showed a dose-sparing effect. At a dosage of 25 µg and higher the serological response became saturated, albeit with a tendency towards higher vaccine-specific T-cell numbers at a dose of 50 µg. Virus neutralisation activity was present after the second vaccination and from a vaccine dose of 25 µg upwards at levels similar to the WHO standard 20/136. Neutralisation activity was broad with about two-fold reduced activity against the beta variant virus. Of note, a more than ten-fold reduction was reported for BNT162b2.[16] MF59 had a positive effect on virus neutralisation. Concentrations of neutralising antibodies against the omicron clade were significantly lower but similar to approved vaccines before updating to omicron BA.1 and BA.5.[17] Whether ABNCoV2 will also need to be updated is currently being investigated in late-stage clinical development of ABNCoV2 (NCT05329220 and NCT05077267). A main advantage of the modular Tag-Catcher-AP205 capsid-like particle vaccine design of ABNCoV2 is the possibility to replace the current vaccine antigen relatively quickly in the event that the SARS-CoV-2 virus should acquire mutations in the RBD domain reducing the efficacy of the ABNCoV2 vaccine. ABNCoV2's tolerability was independent of dose, adjuvant, and time (first *vs* second vaccination). This pattern is similar to other VLP vaccines (eg, against human papillomavirus[18]), whereas mRNA and vectored SARS-CoV-2 vaccines are more reactogenic.[19,20] During the follow-up, two cases of basal cell carcinoma occurred in one participant and were reported as a SUSAR. Basal cell carcinoma is common and its associated mortality is very low.[21] Ultraviolet radiation is the main risk factor for basal cell carcinoma, but it can develop, although rarely, on scar tissue, including vaccination scars. Basal cell carcinoma has been reported after vaccination for smallpox,[22] Bacillus Calmette-Guérin,[23] influenza,[24] typhoid, and hepatitis A.[25] The SUSAR occurred in a participant with a predisposition to skin malignancies and no additional skin anomalies were found, even when actively

screened for. Nevertheless, and despite the low probability of an ABNCoV2-specific causal link, monitoring of late local reactions shall be included as clinical development progresses.

The size and design of the trial, as well as the inclusion of participants in the ongoing national vaccination campaign, precluded measuring the protective efficacy of ABNCoV2. Efficacy in preclinical models has translated reasonably well into humans for other SARS-CoV-2 vaccine candidates.[26] Analogously, promising results from preclinical studies of ABNCoV2, including challenge experiments,[13] as well as antibody levels and consistent virus neutralisation (as a proxy of protection[27]) in the current trial, advocate for ongoing clinical development of this vaccine candidate. Antibody responses induced by ABNCoV2 were in the same range as those induced after two doses of BNT162b2 and responses were boosted to peak levels in individuals receiving a heterologous vaccine (shown in figure 3C–D).[28]

Two participants who received 25 µg ABNCoV2 tested positive for SARS-CoV-2, 16 and 20 weeks after the second vaccination. Both participants had received one dose of BNT162b2 before the SARS-CoV-2 infection, with one participant receiving the dose 9 weeks before and the other 12 weeks before vaccination. These participants had moderate COVID-19-related symptoms, and one of them developed a grade 3 (39·0°C) fever. Whole-genome sequencing revealed delta variant (B.1.617) sub-lineage AY.122 for one and no result for the other participant, in whom viral load was very low. Of note, ten participants remained SARS-CoV-2 negative despite high-risk exposures; five who only received ABNCoV2 and five with at least one other vaccination.

The predefined immunogenicity criterion of our escalation design was based on primary data from previous trials with soluble protein vaccine candidates,[29,30] showing that antibody response following first vaccination had discriminatory power for dose selection. Additionally, the target product profile of WHO included immunogenicity following one vaccination.[31] This approach turned out not to be optimal, as, after one vaccination, the response was low, variable, and did not predict response following second vaccination well. Hence, two immunisations are, at minimum, required for ABNCoV2 in vaccinees who are naive to SARS-CoV-2. cVLPs structurally resemble native viruses and can be highly immunogenic, in particular due to their size, which enables them to be drained directly to the lymph nodes, and their repetitive surface epitope display.[6] cVLPs overcome risks of highly effective live

attenuated vaccines (eg, vaccine-induced disease or reversion) but their immunogenicity is comparable. With ABNCoV2, we observed a dose-sparing effect that saturated at 25 µg when MF59 was added, which might be beneficial for large-scale use. Implementation will also be facilitated after development of formulations of ABNCoV2 with less stringent storage requirements from freezer to room temperature, particularly for its use in remote areas or regions with ineffective health infrastructures.

This trial had several limitations. The durability of the immune responses could not be measured in most participants as nearly all (43 of 45) received a licensed SARS-CoV-2 vaccine during the follow-up of the trial; the study population of mostly young healthy adults was not representative of the population most in need of second-generation SARS-CoV-2 vaccines; the trial was not powered to measure efficacy against infection or disease; only a relatively small set of regimens was tested; and there was no control group with a licensed vaccine, as there were no licensed vaccines available at the start of the trial.

In conclusion, the results of this trial show that ABNCoV2 was well tolerated and induced strong virus neutralising antibody responses after the second vaccination in healthy adults who are naive to SARS-CoV-2. The protein-based ABNCoV2 vaccine is not expected to require ultra-cold storage conditions (−20 and −70°C), as opposed to currently approved mRNA-based COVID-19 vaccines, easing global distribution. These findings support additional clinical development of ABNCoV2 as a second-generation vaccine and show the potential of the modular cVLP platform.

COUGH-1 study group

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Contributors

MJS, MBBM, MAN, ME, and BGM conceived the study and developed the study protocol. WAdJ, MAN, AFS, and BGM acquired funding. MBBM and BGM provided oversight and supervision. HFW was the sponsor's representative of the trial. CF, CH, and RF did the antibody assays. MI, SRP, APU, AB, SR, and JB did the virus neutralisation assays. DSJ, SJ, TL, and OL-A (COUGH-1 trial study group) did the T-cell analysis. BGM did the statistical analysis. MJS and KT (COUGH-1 trial study group), MBPAA, MBBM, and BGM coordinated the trial and oversaw data collection and management. MJS, AFS, MBPAA, CF, CH, RF, WAdJ, MBBM, MAN, and BGM accessed and verified the data. MJS, AFS, MBPAA, CF, CH, RF, MI, WAdJ, MBBM, MAN, and BGM analysed the data. MJS, AFS, MBPAA, CF, MAN, and BGM wrote the original draft of the manuscript. CH, RF, ME, PGK, RtH, HFW, MI, SRP, APU, AB, SR, JB, MS, SME, TG, SC, TGT, AS, MH, and WAdJ, and RD, LG, TMH, CJ, DSJ, SJ, PKK, AK, TL, OL-A, KT, and EV-C (COUGH-1 trial study group) reviewed the manuscript. All authors had access to the data in the study and had final responsibility for the decision to submit for publication.

Data sharing

Deidentified participant data that underlie the results reported in this Article will be made available on request. Proposals should be directed to the corresponding author, benjamin.mordmueller@radboudumc.nl. Proposals will be reviewed and approved by the sponsor on the basis of compliance with the informed consent and scientific merit. After approval of a proposal, data requesters will need to sign a data access agreement. Data can be requested indefinitely.

Declaration of interests

MAN, AFS, AS, TGT, and CJ are listed as coinventors on a patent application covering the AP205 CLP vaccine platform technology (WO2016112921 A1) licensed to AdaptVac. CF, AFS, and WAdJ are employees at AdaptVac, a company commercialising virus-like particle display technology and vaccines, including several patents.

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References

1. WHO. WHO COVID-19 dashboard. https://covid19.who.int/. Date accessed: July 9, 2022.
2. Kasis A, Timotheou S, Monshizadeh N, et al. Optimal intervention strategies to mitigate the COVID-19 pandemic effects. Sci Rep 2022; 12: 6124.
3. Jara A, Undurraga EA, Zubizarreta JR, et al. Effectiveness of homologous and heterologous booster doses for an inactivated SARS-CoV-2 vaccine: a large-scale prospective cohort study. Lancet Glob Health 2022; 10: e798–806.
4. Fougeroux C, Goksøyr L, Idorn M, et al. Capsid-like particles decorated with the SARS-CoV-2 receptor-binding domain elicit strong virus neutralization activity. Nat Commun 2021; 12: 324.
5. Zakeri B, Fierer JO, Celik E, et al. Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. Proc Natl Acad Sci USA 2012; 109: E690–7.
6. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol 2010; 10: 787–96.
7. De Vincenzo R, Conte C, Ricci C, et al. Long-term efficacy and safety of human papillomavirus vaccination. Int J Womens Health 2014; 6: 999–1010.
8. Gao Y, Chen Y, Liu M, et al. Impacts of immunosuppression and immunodeficiency on COVID-19: a systematic review and meta-analysis. J Infect 2020; 81: e93–5.
9. Bonanad C, García-Blas S, Tarazona-Santabalbina F, et al. The effect of age on mortality in patients with COVID-19: a meta-analysis with 611,583 subjects. J Am Med Dir Assoc 2020; 21: 915–8.
10. Ko E-J, Kang S-M. Immunology and efficacy of MF59-adjuvanted vaccines. Hum Vaccin Immunother 2018; 14: 3041–5.
11. Heinzel C, Pinilla YT, Elsner K, et al. Non-invasive antibody assessment in saliva to determine SARS-CoV-2 exposure in young children. Front Immunol 2021; 12: 753435.
12. Underwood AP, Sølund C, Fernandez-Antunez C, et al. Neutralisation titres against SARS-CoV-2 are sustained 6 months after onset of symptoms in individuals with mild COVID-19. EBioMedicine 2021; 71: 103519.
13. Volkmann A, Koopman G, Mooij P, et al. A capsid virus-like particle-based SARS-CoV-2 vaccine induces high levels of antibodies and protects rhesus macaques. Front Immunol 2022; 13: 857440.
14. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 1988; 124: 869–71.
15. Sattler A, Angermair S, Stockmann H, et al. SARS-CoV-2-specific T cell responses and correlations with COVID-19 patient predisposition. J Clin Invest 2020; 130: 6477–89.
16. Planas D, Veyer D, Baidaliuk A, et al. Reduced sensitivity of SARS-CoV-2 variant delta to antibody neutralization. Nature 2021; 596: 276–80.
17. Tan C-W, Lim B-L, Young BE, et al. Comparative neutralisation profile of SARS-CoV-2 omicron subvariants BA.2.75 and BA.5. Lancet Microbe 2022; 3: e898.
18. Schiller JT, Castellsagué X, Garland SM. A review of clinical trials of human papillomavirus prophylactic vaccines. Vaccine 2012; 30: F123–38.

19. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021; 397: 99–111.
20. Chapin-Bardales J, Gee J, Myers T. Reactogenicity following receipt of mRNA-based COVID-19 vaccines. JAMA 2021; 325: 2201–2.
21. Miller DL, Weinstock MA. Nonmelanoma skin cancer in the United States: incidence. J Am Acad Dermatol 1994; 30: 774–8.
22. Rich JD, Shesol BF, Horne DW. Basal cell carcinoma arising in a smallpox vaccination site. J Clin Pathol 1980; 33: 134–5.
23. Ben-Hur N, Avni J, Neuman Z. Basal cell carcinoma following BCG vaccination. Report of two cases. Dis Chest 1963; 44: 653–5.
24. Pace A, Degaetano J. Basal cell carcinoma developing in an influenza vaccine scar. Australas J Dermatol 2004; 45: 75–6.
25. Smith VH, Soon C, Dharma B, et al. Basal cell carcinomas arising in travel vaccination scars. Clin Exp Dermatol 2008; 33: 515–6.
26. Moore JP, Klasse PJ. COVID-19 vaccines: "warp speed" needs mind melds, not warped minds. J Virol 2020; 94: e01083–20.
27. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021; 27: 1205–11.
28. Walsh EE, Frenck RW, Falsey AR, et al. Safety and immunogenicity of two RNA-based COVID-19 vaccine candidates. N Engl J Med 2020; 383: 2439–50.
29. Esen M, Kremsner PG, Schleucher R, et al. Safety and immunogenicity of GMZ2–a MSP3-GLURP fusion protein malaria vaccine candidate. Vaccine 2009; 27: 6862–8.
30. Mordmüller B, Sulyok M, Egger-Adam D, et al. First-in-human, randomized, double-blind clinical trial of differentially adjuvanted PAMVAC, a vaccine candidate to prevent pregnancy-associated malaria. Clin Infect Dis 2019; 69: 1509–16.
31. WHO. WHO target product profiles for covid-19 vaccines, version 3. https://www.who.int/publications/m/item/who-target-product-profiles-for-covid-19-vaccines. Date: April 29, 2020. Date accessed: April 29, 2020.

Supplementary Material

Please scan the QR code below to access the supplementary material for Chapter 8:

CHAPTER 9

General Discussion

Introduction to the discussion

Combatting malaria transmission

Nearly half the world's population lives in areas at risk of malaria transmission.[1] While progress has been made in reducing the number of malaria-endemic countries–from 108 in 2000 to 83 in 2023–the global burden remains high, with an estimated 263 million malaria cases in 2023.[2] And even though mortality rates have steadily declined, 73.7% of those who die from malaria are children under the age of five.[2] This means that in 2023, on average, every hour 50 children under five died from malaria.

The importance of eliminating malaria is not only a matter of improving public health, but it also has economic and social benefits, such as reducing poverty, supporting economic growth, and addressing inequalities. The complexity of malaria as a global health issue stems from the parasite's diversity, socioeconomic disparities, and biological threats to malaria interventions. These threats include (1) deletions in *pfhrp2/3* genes,[3] that allow the parasite to evade detection by most rapid diagnostic tests,[4] (2) vector resistance to insecticides,[3] (3) the emergence and spread of artemisinin partial resistance,[5-8] (4) the threat of resistance to artemisinin-combination therapy (ACT) partner drugs,[9,10] and (5) the invasion and spread of *Anopheles stephensi*,[3,11] a vector that thrives in urban settings. Current vectors prefer rural settings, but given rapid urbanisation in many African regions, the spread of *An. stephensi* increases the risk of malaria transmission. In addition to these biological challenges, ensuring access to adequate treatment and effective vector control remains a major hurdle, as gaps in healthcare infrastructure, supply chain limitations, and insufficient coverage of interventions continue to hinder malaria elimination efforts.

Over the past few decades, malaria researchers have not been idle. Efforts to combat malaria have led to the development, implementation, and advancements of a diverse and integrated set of tools for vector control, diagnosis, treatment, chemoprevention, and vaccination. These tools, while already effective in many contexts, must be continually adapted, implemented effectively, and enhanced to meet the evolving challenges posed by the malaria parasite. A multifaceted approach combining established tools with emerging innovations, will be essential to overcome the challenges posed by this parasite.

One of the hurdles in malaria control is the highly efficient process of *Plasmodium* transmission from man-to-mosquito. Factors such as the prolonged duration of asymptomatic *P. falciparum* infections in humans, mosquitoes feeding on multiple hosts, and the efficient transfer of parasites between humans and mosquitoes contribute to reproduction rates for malaria exceeding 100 in many African regions.[12] While the reproduction number of malaria is a simplification, as *P. falciparum* malaria is endemic and transmission varies due to spatial and temporal factors–such as intervention coverage, naturally acquired or vaccine-induced partial immunity, and the seasonal peaks and drops in vector numbers–[13] this number highlights the important role of transmission in driving disease burden. It also underscores the potential benefits of developing tools to block malaria transmission. In my thesis, we explored several ways to block *P. falciparum* malaria transmission: we tested the transmission-reducing efficacy of existing and new combinations of antimalarials in **Chapters 2, 3A, and 4**, aimed to make advances in *P. falciparum* transmission-blocking vaccine development in **Chapter 5**, and tested a monoclonal antibody to reduce *P. falciparum* transmission in **Chapter 6**.

Sustainability in malaria research

Effective malaria control also requires cross-cutting climate-responsive health strategies to mitigate the impacts of climate change. Climate change is increasingly affecting malaria transmission and disrupting health services, particularly as extreme weather events become more frequent in malaria endemic regions.[14] Environmental and climatic factors play a role in hindering progress towards malaria elimination. An estimated 3.3–3.6 billion people live in areas vulnerable to climate change, particularly in Africa, Asia, Central and South America, and small island states.[15] Climate-related extreme events, such as cyclones, heavy rains and flooding, create ideal breeding conditions for *Anopheles* mosquitoes. These events also disrupt infrastructure, limit access to health care, increase food insecurity and malnutrition, and complicate the delivery of malaria interventions. These factors compound malaria risk and exacerbate disease severity and mortality, as seen in Pakistan after the 2022 floods where a fivefold increase in malaria cases was seen compared with the previous year.[1] Over the next decade, climate impacts are expected to intensify. These effects will disproportionately affect communities in situations of vulnerability who have historically contributed the least to climate change.[15]

Growing attention to addressing climate change has motivated efforts to integrate sustainability into our clinical research practices. In **Chapter 3B**,

we performed a life cycle assessment (LCA) of the clinical trial described in **Chapter 3A** with the aim of informing and supporting sustainable research practices. An LCA is a methodological tool used to quantitatively analyse the life cycle of products/activities within the context of environmental impact. In an LCA, the total life cycle of a product or activity is considered, from the extraction of resource materials to the waste and waste treatment stage, also referred to as cradle-to-grave. The environmental impact of clinical trials on the African continent are yet to be explored. Given differences in study logistics (e.g. material importation, international travel) and climatic conditions (that in turn impact electricity use), trials on the African continent may have markedly different environmental impacts. They may also have different contributors to these impacts compared to studies conducted in Europe or the United States. In **Chapter 3B** we conducted the first comprehensive life cycle assessment of a clinical trial, examining the trial's environmental impact beyond its carbon footprint alone.

From malaria to COVID-19: a brief research detour

The COVID-19 pandemic disrupted many aspects of daily life and research, forcing all our malaria clinical trials to be put on hold. At the same time, the high demand for COVID-19 vaccines led to substantial funding and collaborative efforts that accelerated vaccine development across multiple platforms. Among the first to reach the market were mRNA vaccines, such as Pfizer/BioNTech's Comirnaty®.[16,17] Amid this shifting scientific landscape, we decided to test, for the first time, a novel modular vaccine platform that was originally developed to improve malaria vaccines. As a result, we conducted a first-in-human trial of a novel COVID-19 vaccine: ABNCoV2 (**Chapter 8**). This capsid virus-like particle (cVLP) vaccine entered the vaccine development race later due to its more complex design and development requirements. In ABNCoV2 a novel VLP technology that allows covalent, directional, high density binding of different proteins on the VLP surface was used.[18] The findings from our phase 1 trial were promising, demonstrating a robust immune response with dose-dependent antibody titres that plateaued at a vaccination dose of 25-70 µg ABNCoV2. Following the second vaccination, live virus neutralisation activity was strong, with PRNT50 titres comparable to the WHO20/136 standard—showing sustained activity against the Alpha and Delta variants, and a modest reduction against the Beta variant. Neutralisation was lower against the Omicron (BA.1) variant, consistent with approved vaccines before improvements in vaccine design to better cover Omicron BA.1 and BA.5.[19]

Our trial involved a staggered dose-escalation of ABNCoV2, alongside adjuvant selection, to inform future studies on optimal dosage and the potential inclusion of an adjuvant. The decision on whether to proceed with or without the MF59 adjuvant was predefined and based on data from the first three groups (up to 25 µg ABNCoV2), collected two weeks after the first vaccination. This timepoint was selected based on previous trials with soluble protein vaccine candidates that suggested that antibody responses after the first vaccination had discriminatory power for dose selection.[20-22] This aligned with the World Health Organization (WHO) target product profile for COVID-19 vaccines at that time, that strongly favoured single-dose formulations.[23] However, after one vaccination, we observed that the response was low and heterogeneous, and did not predict response following second vaccination well. We observed that IgG antibody responses peaked two weeks after the second ABNCoV2 vaccination. In hindsight, a more informed decision on the inclusion of the adjuvant could have been made based on the data collected two weeks following the second dose, as we found that MF59 adjuvant had a positive effect on both IgG responses and virus neutralisation.

The results of our phase 1 trial in **Chapter 8** lead to the advancement of ABNCoV2 to later-stage trials. In the phase 2 study, ABNCoV2 successfully boosted neutralising antibody levels against several SARS-CoV-2 variants, including Alpha, Beta, Delta, and Omicron BA.4/5, reaching efficacy levels exceeding 90%.[24] Moreover, a follow-up study showed that 12 months after the booster, these antibody levels remained high,[24] suggesting that sustained protection may be attainable. Phase 3 studies further confirmed the vaccine's safety and ability to induce immunity comparable to the widely used Comirnaty® vaccine.[25] However, as the pandemic evolved, the vaccine's slower adaptability to emerging variants reduced its commercial viability.[26] While ABNCoV2 has not achieved the same commercial success as other SARS-CoV-2 vaccines and played no role in strategies to control the pandemic, the platform's proven ability to elicit strong, long-lasting immunity remains important for future vaccines, including a role in vaccination during potential future pandemics.

The potential of cVLP vaccine technology

The lessons learnt in **Chapter 8** are valuable as this platform can be leveraged for vaccines beyond COVID-19. The cVLP platform presents antigens in a dense, repetitive array that mimics the structure of viruses, thereby enhancing B cell activation and improving antibody responses.[27] This technology is particularly

attractive for antigens that are otherwise poorly immunogenic, making it also relevant for vaccine targets like those in malaria. One of those vaccines is the placental malaria vaccine PAMVAC. In an effort to improve its immunogenicity, a cVLP version of PAMVAC is being developed,[28] which is scheduled for clinical testing in 2025.[29] *P. falciparum* provides a particularly interesting model for this vaccination approach, as its multi-stage lifecycle allows for the study and targeting of distinct parasite stages. This contrasts with many other infectious diseases where the same pathogen stage is responsible for both infection and transmission. Beyond malaria, the cVLP platform is also being explored for vaccines against other infectious diseases, including Nipah, Marburg, and Sudan viruses.[30] Current efforts primarily focus on the Nipah virus, aiming to generate clinical proof-of-concept data within the coming years for this high-fatality zoonotic pathogen that has no approved vaccines to date.[31] These efforts illustrate how cVLP technology, initially deployed in response to COVID-19 in **Chapter 8**, is broadening its relevance beyond a single disease.

The early stages of the COVID-19 pandemic also demonstrated public acceptance of a vaccine that was largely administered to low-risk population to protect vulnerable individuals—an important consideration for *P. falciparum* transmission-reducing tools, which similarly rely on 'altruistic behaviour' or intervention acceptance for the greater good, as those receiving them do not directly benefit.

Unveiling the transmission-reducing potential of antimalarials

The primary objective in malaria treatment is to clear pathogenic asexual blood-stage parasites. The WHO currently recommends six different artemisinin-based combination therapies (ACTs) as first- and second-line treatments for uncomplicated *P. falciparum* malaria.[32] Artemisinin and its derivates, derived from the plant *Artemisia annua*, are potent medications known for their ability to rapidly reduce the number of *Plasmodium* parasites in the bloodstream.[33] ACTs pair an artemisinin derivative (such as artemether, dihydroartemisinin, or artesunate) with a partner drug (such as lumefantrine, piperaquine, or amodiaquine). The artemisinin compound acts to rapidly decrease the parasite load during the first three days of treatment, while the partner drug eliminates the remaining parasites to cure the infection and, depending on the drug used, may provide a period of effective prophylaxis.

ACTs help prevent the development and spread of resistance to the individual drugs by combining two drugs with different mechanisms of action.[34] However, if the partner drug levels fall below the therapeutic threshold, it may provide a selective window for parasites with reduced drug sensitivity to survive. Therefore, the pharmacokinetic profiles of both drugs must be balanced.

ACTs are highly effective against asexual parasites.[35] Although ACTs generally reduce the gametocyte density more than non-ACTs,[36] significant variation exists in their gametocyte-clearing efficacy.[37] Importantly, artemisinin partner drugs also exhibit limited activity against mature *P. falciparum* gametocytes at the concentrations achieved in clinical practice.[22,38-40] However, a disconnect may exist between gametocyte presence and infectivity. Gametocytes can infect mosquitoes even at sub-microscopic densities,[41] meaning that their apparent absence does not rule out infectivity. On the other hand, some antimalarials may also inhibit parasite viability after mosquito ingestion[22,42] or sterilise gametocytes, rendering them non-infectious despite being detectable by microscopy or molecular (RNA-based) methods.[43] Thus, gametocyte quantification alone may not accurately reflect transmission potential. Mosquito feeding assays can provide more reliable information on post-treatment transmissibility.[35]

Artemether-lumefantrine: the most widely used ACT

Artemether-lumefantrine is by far the most widely used ACT and accounts for over 80% of all treatments for uncomplicated malaria.[44] Given its widescale use, it is striking that uncertainty exists about its effect on transmission. The sparse studies done to address this have yielded inconsistent results. Among the studies that conducted mosquito feeding assays after artemether-lumefantrine treatment, three studies from Kenya and Tanzania reported detectable transmission between 7 and 14 days after treatment with either artemether-lumefantrine alone[45,46] or in combination with primaquine.[47] In contrast, two studies conducted in Burkina-Faso and The Gambia found near-complete abrogation of transmission one week after artemether-lumefantrine treatment.[48,49] One possible explanation for these regional discrepancies could be the emergence of *P. falciparum* strains in East Africa with subtly reduced susceptibility to ACTs. However, these studies were conducted before the emergence of artemisinin resistance in Africa, suggesting that artemisinin resistance is unlikely to explain the observed differences. Another explanation is variability in drug absorption among individuals, which can influence the efficacy of artemether-lumefantrine. Factors such as food intake, co-

administered medications, and adherence to dosing schedules can affect drug bioavailability, and consequently, its gametocytocidal activity. While these strains may not yet exhibit significant effects on asexual parasite clearance, they could possess a lowered sensitivity to the gametocytocidal effects on artemether-lumefantrine. This reduced sensitivity might allow gametocytes to persist longer post-treatment, thereby maintaining the potential for transmission. The only study that assessed transmission to mosquitoes both before and after artemether-lumefantrine treatment, prior to the work conducted in this thesis, found that only one (2%) of 49 individuals infected mosquitoes on day 7 post-treatment with artemether-lumefantrine, with mosquito infection rates significantly lower than those observed before treatment.[49] These conflicting results highlighted the need for further investigation of the transmission reducing effect of the most widely used ACT worldwide.

In **Chapter 3A**, we assessed the efficacy of artemether-lumefantrine with and without a single low dose of primaquine. The aim was to evaluate its effect on reducing gametocyte carriage and transmission to mosquitoes in Malian individuals, as measured by mosquito feeding assays. Our results show that in individuals who were infectious before treatment, the median percentage reduction in mosquito infection rate 2 days after treatment was 100% (p=0.0011) with artemether-lumefantrine and 100% (p=0.0001) with artemether-lumefantrine plus a single low dose of primaquine. Only 2 out of 19 individuals who were infectious at baseline continued to infect mosquitoes on day 2 after receiving artemether-lumefantrine, and none did so on day 5. No mosquito infections were observed after day 5 in either artemether-lumefantrine treatment groups.

These findings demonstrate the effectiveness of artemether-lumefantrine in preventing nearly all mosquito infections in Malian individuals. With our study design that specifically recruited high-density, highly infectious gametocyte carriers, we consider this conclusive evidence for the high impact of artemether-lumefantrine on transmission in settings unaffected by artemisinin or lumefantrine resistance. While the addition of a gametocytocidal drug to artemether-lumefantrine may not offer significant added benefit in Mali, it could be valuable when used with other ACTs or in other malaria-endemic regions. In routine care settings, where only the first dose of artemether-lumefantrine is typically observed, adherence to the full artemether-lumefantrine regimen may be suboptimal. This could allow gametocytes to

persist longer, thereby increasing the potential benefit of gametocytocide co-treatment. Our data thus only provide insight into the impact of artemether-lumefantrine under optimal conditions where treatment is directly observed.

The potent transmission-blocking effect of artemether-lumefantrine in **Chapter 3A** contrasts with what we saw in **Chapter 2** with the ACT dihydroartemisinin-piperaquine, that resulted in a reduction of mosquito infection rates only 7-14 days after treatment. Similarly, another study conducted at the same facilities showed that the ACT pyronaridine-artesunate treatment led to significant reductions in mosquito infection rates, but only 10 days post-treatment.[50] Furthermore, unpublished data from an ongoing trial in Uganda suggests that the efficacy of artemether-lumefantrine in reducing gametocyte carriage and transmission to mosquitoes is lower than what we observed in **Chapter 3A**. In this Ugandan trial, 3 out of 18 participants remained infectious on day 2, and 1 out of 17 was still infectious on day 7 after treatment (NCT06347471). Additionally, an ongoing study in Ethiopia has shown transmission occurring even 14 days after artemether-lumefantrine treatment (NCT04241705). While our findings confirm the effectiveness of artemether-lumefantrine in Mali, it is important to recognise that this may not be the case in all malaria-endemic regions and can change over time. It is therefore important to regular monitor the transmission-reducing efficacy of antimalarials.

Addition of the long-acting 8-aminoquinoline gametocytocidal drug tafenoquine

The WHO recommends the use of a single low-dose of the 8-aminoquinoline drug primaquine in addition to ACT in areas with low transmission and areas facing artemisinin resistance.[51] Although primaquine is primarily indicated for the radical cure of *P. vivax* and *P. ovale* malaria by eliminating dormant liver-stage hypnozoites, it also serves as a *P. falciparum* gametocytocide. However, its short half-life of 4-9 hours might prevent it from affecting any gametocytes that arise late during infections or develop after recrudescence. Additionally, during the initial 2-3 days after stage V gametocytes enter circulation, these immature forms may exhibit reduced susceptibility to primaquine's gametocytocidal effects. Given that ACTs primarily target asexual stage parasites and early-stage gametocytes, a potential window during early stage V maturation exists where gametocytes are less susceptible to both treatments. Consequently, the effect of a single low-dose of primaquine is likely to be limited to the sterilisation of currently circulating gametocytes.[52]

Furthermore, primaquine's efficacy can be compromised in individuals with reduced CYP2D6 activity, as it requires metabolic activation by this enzyme.[53] In contrast, tafenoquine–primaquine's long-acting analogue–has been approved as a single-dose radical cure for and prophylaxis against *P. vivax* infection. Tafenoquine does not appear to depend on CYP2D6 for its activation, potentially offering more consistent gametocytocidal activity across diverse populations.[54] Its long half-life of approximately 15 days could provide an advantage over short-lived gametocytocidal treatment by maintaining therapeutic levels that could target gametocytes emerging later in the infection.[55]

In **Chapter 2**, we conducted the first assessment of the *P. falciparum* gametocytocidal and transmission-reducing properties of different doses of tafenoquine (0.42 mg/kg, 0.83 mg/kg, or 1.66 mg/kg) in combination with dihydroartemisinin-piperaquine. Dihydroartemisinin-piperaquine is an ACT used for the treatment of uncomplicated *P. falciparum* malaria, mass drug administration (MDA), and is also considered as an alternative antimalarial for seasonal malaria chemoprevention (SMC), having demonstrated non-inferiority to sulfadoxine-pyrimethamine plus amodiaquine in Uganda.[56] In our study, we showed that tafenoquine was well tolerated at all doses and expedited gametocyte clearance compared to dihydroartemisinin-piperaquine alone. While feeding assays at day 2 did not reveal significant transmission reduction, by day 7 all the tafenoquine arms achieved a median 100% reduction in transmission–with IQRs of 98.36–100 for the 0.42 mg/kg dose and 100–100 for the 0.83 and 1.66 mg/kg doses (p-values ranging from 0.0001 to 0.0005)–compared to a 79.95% reduction (IQR 57.15–100, p=0.0005) for dihydroartemisinin–piperaquine alone.

The combination of tafenoquine with a non-ACT for clearing *P. falciparum* gametocytes and preventing transmission had not yet been tested prior to the work in this thesis. In **Chapter 3A**, we assessed the efficacy of 1.66 mg/kg tafenoquine in combination with the non-ACT sulfadoxine-pyrimethamine plus amodiaquine for reducing gametocyte carriage and transmission. Sulfadoxine-pyrimethamine plus amodiaquine is currently the only recommended antimalarial for SMC. In 2024, SMC was administered to approximately 54 million children across eligible regions.[57] Previous studies have shown significant post-treatment transmission potential following the administration of sulfadoxine-pyrimethamine plus amodiaquine, with infectivity and mosquito infection rates remaining unaffected for the first 7 days and reductions in gametocytaemia only observable after 28 days.[45,58] The increased gametocyte densities in the first two weeks after sulfadoxine-pyrimethamine plus

amodiaquine treatment may therefore limit the effectiveness of SMC in reducing transmission,[46] and thereby limit the potential community benefit that can be achieved by SMC. Our results in **Chapter 3A** demonstrate that a single low dose of 1.66 mg/kg tafenoquine effectively blocks transmission within 7 days. In contrast, the median percentage reduction in mosquito infection rate 7 days after treatment with sulfadoxine–pyrimethamine plus amodiaquine was only 64%. While we observed tafenoquine's efficacy in reducing *P. falciparum* transmission, the duration of its gametocytocidal and transmission-blocking effect remains unknown. Future trials are required to examine the duration of its *P. falciparum* gametocytocidal activity.

Although tafenoquine is likely to have a transmission-blocking effect that is longer than that of primaquine, its wider adoption is limited by concerns about potentially sustained haemolysis in G6PD-deficient individuals.[59] Testing for G6PD deficiency is currently required before administrating tafenoquine in dosing schedules intended for the treatment of *P. vivax* (ie, 200 mg/day for 3 days or a single dose of 300 mg).[55] In **Chapters 2 and 3A**, only individuals with normal G6PD levels were included in the trial to ensure comparability. However, the global distribution of G6PD deficiency mirrors that of *P. falciparum*.[60] It is therefore important to determine the minimally efficacious dose and assess the safety thereof in G6PD-deficient individuals. The highest dose of tafenoquine that we tested in **Chapters 2 and 3A** was 1.66 mg/kg, equivalent to approximately 100 mg for an individual of 60 kg. Longitudinal safety data on low-dose tafenoquine in G6PD-deficient individuals, similar to the data collected for low-dose primaquine (for which G6PD testing is no longer required),[61,62] will be needed before tafenoquine can be more widely adopted.

The efficacy of an antimalarial is dependent on multiple factors, including storage conditions, administration (e.g. with a fatty meal or on an empty stomach), adherence to the regimen, and the presence of emerging resistance. In this context, tafenoquine may have a valuable role in future malaria control strategies–provided that a single low dose is confirmed to be both effective and safe in individuals with G6PD deficiency. Although tafenoquine exhibits slower onset of transmission-blocking activity than primaquine, which blocks transmission within 5 days, its extended duration of action offers advantages. This extended activity may be relevant for targeting drug-resistant infections, where gametocytes are more likely to persist. For example, *pfkelch13* mutant parasites may preferentially survive artemisinin exposure and infect mosquitoes.[63] Moreover, slow asexual parasite clearance is associated with

prolonged gametocyte production,[64] and recrudescent infections following ACT treatment may generate gametocytes that are unaffected by the short-acting artemisinins and primaquine. In such cases, tafenoquine's long half-life could help eliminate gametocytes emerging after treatment, including those from partially resistant parasites. As such, single low dose of tafenoquine holds potential not only for enhancing individual-level transmission blocking, but also for curbing the spread of drug-resistant malaria and this potential may be greater than that of primaquine. Future research should confirm tafenoquine's safety profile in G6PD-deficient populations and determine the optimal dosing strategy to maximise both efficacy and reach.

Pooled analysis of antimalarials

Individual clinical trials are often insufficient to allow all policy-relevant comparisons and analyses. Standard treatment efficacy studies have benefited from a harmonised protocol across settings that allows a comparison of the efficacy of antimalarial drugs in different parasite populations and at different time-points. A similar standardisation is considerably more complex for studies that aim to quantify the transmission-blocking effects of antimalarials since assays are harder to standardise[65] and far fewer sites have transmission facilities. A much smaller body of evidence is therefore available. In **Chapters 2, 3A, and 4**, the sample size of individual trials was designed to assess change in infectivity within treatment arms, not compare transmission reducing effects between treatment arms. In a first attempt to comprehensively compare the transmission-reducing effects of different antimalarials and combinations, we conducted a pooled analysis of individual-level data from six clinical trials, including data from **Chapters 2, 3A, and 4**.[66] All these six trials measured mosquito transmission endpoints in addition to performing sensitive gametocyte quantification, and were conducted using the same mosquito feeding protocols and assays in Ouélessébougou, Mali, between 2013 and 2023.[50,58,67-70]

The pooled analysis of 422 participants from these six studies, treated with 15 antimalarial regimens, demonstrated differences in gametocytocidal and transmission-blocking activity among the various ACTs and the non-ACT sulfadoxine-pyrimethamine plus amodiaquine. The addition of single low dose primaquine accelerated gametocyte clearance and led to a substantially greater reduction in mosquito infection rate within 48-hours of treatment for each ACT, while a single low dose of tafenoquine showed a delayed but

effective response compared to primaquine.[66] By day 7, reductions from baseline were observed at 68.42% (95% CI: 21.76% to 87.25%, p=0.0128) in the non-ACT with primaquine group, 90.57% (95% CI: 82.08% to 95.03%, p<0.0001) in the ACT with primaquine group, and 61.54% (95% CI: 30.38% to 79.75%, p=0.0016) in the ACT with tafenoquine group (**Figure 1**).

Notably, artemether-lumefantrine, tested in **Chapters 3A and 4**, was significantly more effective in reducing mosquito infection rates within 48 hours than dihydroartemisinin-piperaquine (p=0.0158; **Chapter 2**) and sulfadoxine-pyrimethamine plus amodiaquine (p=0.0442; **Chapter 3A**). Artemether-lumefantrine performed near-significantly better compared to pyronaridine-artesunate (p=0.0510) and artesunate-amodiaquine (p=0.0777; **Chapter 4**) (**Figure 2A**). By day 7 after treatment, the reductions in infectivity became more pronounced across most treatments (**Figure 2B**).

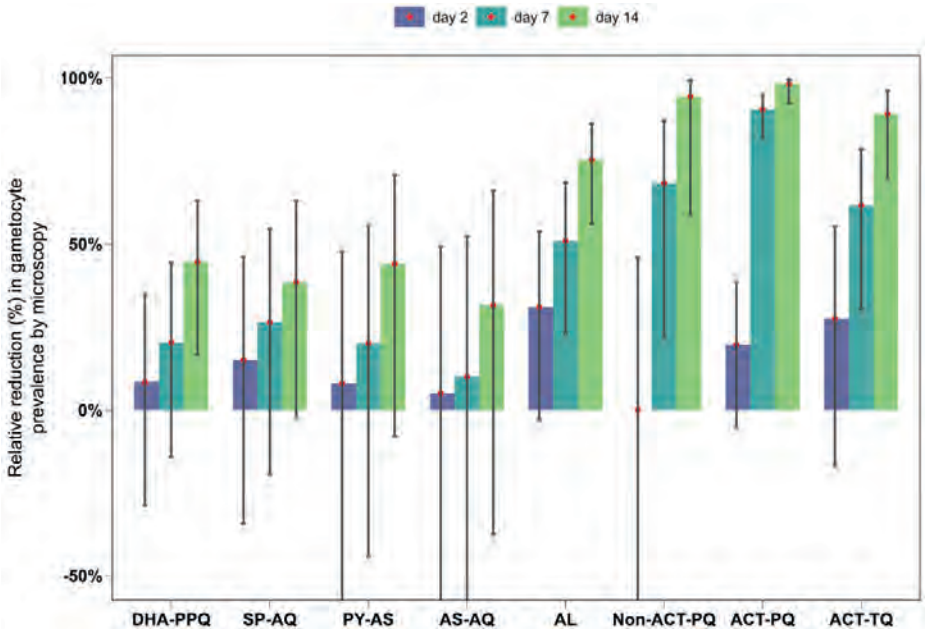

Figure 1. Gametocyte prevalence. Bar charts illustrating the relative reduction compared to baseline in gametocyte prevalence by microscopy, by treatment arm over three time points (Day 2 - indigo, Day 7 - turquoise, and Day 14 - green). Vertical bars depict the 95% confidence intervals for these estimates. Red dots represent observed means. The y-axis was cut off below -50 due to inflated standard errors, as a result of reductions from baseline close to zero or high measurement uncertainty. Visualisations represent data from 422 individuals at baseline, 369 individuals at day 2, 357 individuals at day 7 and 357 individuals at day 14. DHA-PPQ = dihydroartemisinin-piperaquine; SP-AQ = sulfadoxine-pyrimethamine plus amodiaquine; PY-AS = pyronaridine-artesunate; AS-AQ = artesunate-amodiaquine; AL = artemether-lumefantrine; PQ = primaquine; TQ = tafenoquine.

A

	DHA-PPQ	SP-AQ	PY-AS	AS-AQ	AL	Non-ACT-PQ	ACT-PQ
ACT-TQ	11.66% (−117.42%, 140.74%), p=0.8595	−1.96% (−159.49%, 155.58%), p=0.9806	−21.65% (−195.41%, 152.11%), p=0.8071	−24.88% (−207.54%, 157.78%), p=0.7895	79.76% (−60.78%, 220.30%), p=0.2660	98.81% (−60.49%, 258.11%), p=0.2241	82.22% (−58.29%, 222.74%), p=0.2514
ACT-PQ	−70.57% (−126.10%, −15.03%), p=0.0128	−84.18% (−164.10%, −4.27%), p=0.0390	−103.88% (−206.09%, −1.66%), p=0.0464	−107.11% (−223.60%, 9.59%), p=0.0720	−2.46% (−4.99%, 0.06%), p=0.0556	16.59% (−82.50%, 115.67%), p=0.7428	
Non-ACT-PQ	−87.15% (−180.51%, 6.20%), p=0.0673	−100.77% (−201.82%, 0.28%), p=0.0506	−120.46% (−262.82%, 21.89%), p=0.0972	−123.69% (−276.78%, 29.40%), p=0.1133	−19.05% (−118.17%, 80.06%), p=0.7064		
AL	−68.10% (−123.69%, −12.51%), p=0.0164	−81.72% (−161.67%, −1.77%), p=0.0451	−101.41% (−203.66%, 0.83%), p=0.0519	−104.64% (−221.36%, 12.08%), p=0.0789			
AS-AQ	36.54% (−92.70%, 165.78%), p=0.5795	22.92% (−118.52%, 164.36%), p=0.7508	3.23% (−151.90%, 158.36%), p=0.9674				
PY-AS	33.31% (−83.01%, 149.63%), p=0.5746	19.69% (−110.05%, 149.44%), p=0.7661					
SP-AQ	13.62% (−76.69%, 103.93%), p=0.7676						

B

	DHA-PPQ	SP-AQ	PY-AS	AS-AQ	AL	Non-ACT-PQ	ACT-PQ
ACT-TQ	−27.55% (−60.22%, 5.13%), p=0.0984	−51.50% (−136.95%, 33.94%), p=0.2374	−75.84% (−189.70%, 38.02%), p=0.1917	−10.84% (−78.74%, 57.05%), p=0.7543	26.68% (−26.77%, 80.14%), p=0.3279	54.47% (−28.13%, 137.07%), p=0.1962	26.61% (−26.84%, 80.07%), p=0.3291
ACT-PQ	−54.16% (−96.47%, −11.86%), p=0.0121	−78.12% (−150.67%, −5.57%), p=0.0348	−102.45% (−202.99%, −1.91%), p=0.0458	−37.46% (−79.33%, 4.41%), p=0.0795	0.07% (−0.76%, 0.89%), p=0.8715	27.85% (−53.48%, 109.19%), p=0.5021	
Non-ACT-PQ	−82.02% (−157.88%, −6.15%), p=0.0341	−105.97% (−194.22%, −17.73%), p=0.0186	−130.31% (−259.62%, −0.99%), p=0.0483	−65.31% (−156.79%, 26.17%), p=0.1617	−27.79% (−109.12%, 53.55%), p=0.5031		
AL	−54.23% (−96.54%, −11.92%), p=0.0120	−78.19% (−150.74%, −5.64%), p=0.0347	−102.52% (−203.06%, −1.98%), p=0.0457	−37.53% (−79.39%, 4.34%), p=0.0790			
AS-AQ	−16.70% (−76.22%, 42.82%), p=0.5823	−40.66% (−124.42%, 43.10%), p=0.3414	−64.99% (−173.90%, 43.91%), p=0.2421				
PY-AS	48.29% (−60.78%, 157.36%), p=0.3855	24.33% (−99.65%, 148.31%), p=0.7005					
SP-AQ	23.96% (−55.00%, 102.91%), p=0.5520						

Absolute difference in relative reduction

0% 20% 40% 60% 80% 100% 120%

Figure 2. Between-group comparison of the reduction in the proportion of infected mosquitoes at day 2 (A) and day 7 (B) post-treatment, compared to baseline. Heatmaps representing the absolute difference between treatment groups in the relative reduction in proportion infected mosquitoes at days 2 (A) and day 7 (B) post-treatment, compared to baseline, with 95% CI and p-values. Heatmap cells are coloured by the absolute difference. For example, the absolute difference between DHA-PPQ and AL in the relative reduction in proportion infected mosquitoes at day 2 is -68.09% (-123.67%, -12.51%), and the difference between these arms (68.09% lower reduction for DHA-PPQ) is statistically significant (p=0.0163). DHA-PPQ = dihydroartemisinin-piperaquine; SP-AQ = sulfadoxine-pyrimethamine plus amodiaquine; PY-AS = pyronaridine-artesunate; AS-AQ = artesunate-amodiaquine; AL = artemether-lumefantrine; PQ = primaquine; TQ = tafenoquine.

The results from this analysis can help inform malaria treatment policies aimed at elimination efforts and can be incorporated into mathematical models to enhance the precision of predictions about community-level transmission and the emergence of drug resistance under different treatment strategies.

Deploying the transmission-reducing potential of antimalarials to combat resistance and maximise community benefits

Community-level benefits of antimalarials combined with single-low dose of 8-aminoquinoline

The WHO malaria policy and advisory group has recommended the broader adoption of single low-dose primaquine in countries where partial resistance has been detected.[12] As evidence grows regarding the transmission-blocking effects of primaquine and tafenoquine in individual gametocyte carriers, community-based trials are needed to formally demonstrate whether reducing transmission shortly after treatment will result in a community benefit. This can help determine the added value of these gametocytocides in first-line antimalarial treatments and mass treatment campaigns aimed at reducing the transmission of (partially drug-resistant) *P. falciparum* parasites. In **Chapters 2, 3A, and 4** high-density gametocyte carriers were included in the trial. By assessing infectivity before and after treatment, our findings offer valuable insights into the transmission-reducing effects of the tested antimalarials in highly infectious individuals. However, to assess the broader community-level benefits of combining antimalarials with 8-aminoquinolines, additional study designs are required. This includes evaluating their use in accordance with WHO guidelines (i.e., at clinical presentation) or in mass treatment campaigns, such as SMC, to reduce transmission at the community level. Possible endpoints of these trials include the number of incident

infections experienced by a community where primaquine or tafenoquine are deployed or the number of wild-caught *P. falciparum*-infected mosquitoes.

Optimising seasonal malaria chemoprevention implementation

SMC with sulfadoxine-pyrimethamine plus amodiaquine is a safe and cost-effective strategy for preventing malaria among young children in areas where the disease is highly seasonal.[57] Children in age groups at high risk of severe malaria are eligible for SMC, typically those under 5 years old in areas with intense seasonal transmission. SMC is currently being implemented in 19 countries across the Sahel and other seasonal regions of sub-Saharan Africa, benefiting increasing numbers of children.[2] The number of children treated per SMC cycle in these countries rose from about 170.000 in 2012 to 53 million in 2023.[2]

However, SMC medication might benefit from improvements in terms of transmission-blockade, as there is still significant transmission after sulfadoxine-pyrimethamine plus amodiaquine treatment. In **Chapter 3A** we observed less than 10% reduction in the percentage of infected mosquitoes on day 10 following treatment. As the only non-artemisinin-based antimalarial recommended for systematic distribution via SMC,[57] the poor performance of sulfadoxine-pyrimethamine plus amodiaquine against gametocytes may limit the overall community impact of the intervention. In addition to considering gametocytocidal compound, it is important to note that current SMC strategies primarily target children under five, which is an age group that does not contribute the most to the infectious reservoir and is therefore unlikely to meaningfully reduce transmission.

Field trials and meta-analyses indicate that extending SMC to older children contributes to reducing the malaria burden, supporting the effectiveness of malaria elimination programs.[71] In June 2022, the WHO introduced updated chemoprevention guidelines, recommending that the number of SMC cycles and the age range of protected children be adapted to local epidemiology, offering greater flexibility to enhance the intervention's reach. While older children are at lower risk of clinical disease and severe malaria in most endemic settings, they are relevant drivers of malaria transmission. School-aged children (aged 5-15 years) carry parasites at higher densities than younger children and for longer periods,[72,73] whilst also being disproportionally bitten by mosquitoes due to their larger body size and lower coverage with insecticide treated nets.[74] This results in a large contribution to the transmission reservoir.[73,75,76] Including

children up to 10 or even 15 years old in SMC campaigns could therefore yield substantial benefits.

The emergence of resistance

Regions of low malaria transmission have generally been the first where resistance to antimalarial drugs has emerged, possibly due to a combination of factors. One factor could be that in populations with low antimalarial immunity, a higher proportion of infected individuals develop symptoms and seek treatment and thus drug pressure on the parasite population is relatively high. Additionally, individuals with lower levels of acquired immunity are less capable of clearing mutant parasites that may be less fit, compared to those with higher levels of immunity. Moreover, low transmission intensity results in fewer polyclonal infections, reducing competition between parasites within a host, which could otherwise be disadvantageous for mutant infections that have experienced a fitness cost.[77] Nonetheless, polyclonal infections, when they do occur, may allow recombination between parasite genotypes in the mosquito midgut, potentially facilitating the acquisition of compensatory mutations that restore fitness. These conditions could allow initially selected resistant parasites to evolve and overcome fitness costs, becoming better adapted and more established.

Furthermore, unstable malaria transmission can lead to temporarily high malaria incidence in populations with low antimalarial immunity, creating favourable conditions for the selection of partial resistance to artemisinins. Partial resistance to artemisinins is characterised by delayed clearance of *P. falciparum* parasites from the bloodstream following treatment with ACTs, typically due to mutations affecting the ring stage of the parasite's life cycle.[78] In northern Uganda, the implementation of a comprehensive indoor residual spraying program drastically reduced the malaria burden. However, its discontinuation led to a major rebound in malaria cases in a population with declining immunity and has potentially driven the selection of partial resistance to artemisinins.[79] Similarly, in Rwanda, western Uganda, and the Horn of Africa, unstable malaria transmission may have allowed high infection rates in populations with low immunity, promoting the emergence and spread of resistance. Notably, malaria transmission is now unstable across much of Africa, with outbreaks following periods of low incidence, potentially facilitating the selection of partial resistance to artemisinins continent-wide.

In regions with unstable malaria transmission and emerging resistance, the role of primaquine (and potentially tafenoquine in the future) in malaria treatment and control could become increasingly important. A single low dose of a gametocytocidal drug in combination with an ACT, as tested in **Chapters 2, 3A and 4**, might offer an effective additional strategy to curb the spread of resistance by eliminating gametocytes and thereby lowering the likelihood that partially resistant parasites are transmitted. To ensure the continued effectiveness of primaquine and prevent the emergence of resistance to this drug as well, careful monitoring and accurate weight-based dosing in accordance with treatment guidelines is required.

The state of artemisinin resistance and its current clinical consequences

The emergence of partial resistance to artemisinin, along with the risk of resistance to ACT partner drugs, poses a significant threat to efforts aimed at reducing the malaria burden. A study using an individual-based malaria transmission model, incorporating data from both Southeast Asia and Africa, estimated the impact of artemisinin and partner drug resistance on malaria morbidity in Africa.[9] The model simulated various resistance scenarios, considering slow parasite clearance and recrudescent infections, to assess their effects on malaria incidence and parasite prevalence. It predicted an additional 78 million malaria cases over a five year period, which is a 7% increase compared to a scenario without resistance. It goes without saying that these predictions have to be interpreted with caution and many factors in addition to drug resistance will influence future malaria burden. A parallel could be drawn with early analyses of the impact of climate change on global malaria burden. Global warming resulted in more regions and longer periods that are suitable for malaria transmission but economic development and widescale deployment of control measures outbalanced this effect and resulted in a net reduction in malaria burden.[80]

The spread of artemisinin partial resistance across the African continent is a growing concern. Surveillance of *pfkelch13* polymorphisms linked to artemisinin partial resistance has been conducted in several countries across the WHO African Region,[3] with evidence of partial resistance linked to *pfkelch13* mutations in Rwanda,[7] Eritrea,[8] Uganda,[6] and Tanzania.[5] Moreover, artemisinin partial resistance has become suspected in Ethiopia and Sudan, and more recently in Namibia and Zambia.

Artemisinin partial resistance is particularly clinically important when artemisinins are used as monotherapy for complicated malaria,[81] and when coinciding with resistance to ACT partner drugs that are used for uncomplicated malaria. Resistance to either component of an ACT can influence the development of resistance to the other. However, the extent and nature of this interaction depend on the specific drug combination and regional factors. Artemisinin partial resistance along with resistance to ACT partner drugs has been linked to high treatment failure rates for key ACTs.[82-84] Most countries rely heavily on artemether-lumefantrine, creating drug pressure and therefore vulnerability to any reduction in its therapeutic efficacy. Preserving the efficacy of lumefantrine is important as it is used in both the triple ACT artemether-lumefantrine plus amodiaquine and in ganaplacide-lumefantrine, which will likely be among the next new antimalarial treatments to become available.[85]

Slowing down the spread of resistance by alternating or combining artemisinin therapies

There are several strategies to limit the transmission of resistant parasites and retain clinical efficacy. One of the strategies includes deploying multiple first-line ACTs. Multiple first-line therapies involve using two or more effective ACTs to treat uncomplicated malaria, either simultaneously or in rotation. This strategy aims to extend the therapeutic lifespan of ACTs with partner drugs exerting different and opposing pressure on parasite populations.[1,86] The choice of strategy for multiple first-line therapies will be determined by the specific context and feasibility within each country.[85] A recent study in Burkina Faso showed that deployment of multiple first-line therapies is operationally feasible and acceptable by stakeholders in the health systems in Burkina Faso.[87] Mathematical modelling could be used to evaluate the potential impact of different options. But ultimately, real-world implementation will reveal whether the strategy might lead to issues such as stockouts, confusion in clinical practice, or reduced adherence among patients having to follow different dosing regimens (e.g. different doses per day or different durations of treatment).

Another promising strategy is the use of triple artemisinin-based combination therapies (TACTs) that combine an ACT with a second partner drug. This approach may involve administering all three drugs concurrently over a standard three-day course or extending the treatment duration by sequential administration of two different ACTs over six days. Parasites are less likely

to acquire mutations conferring resistance to both drugs within a short period. The choice between these regimens depends on factors such as drug pharmacokinetics, patient adherence, and the specific resistance pattern in the region. Different drugs can exert distinct selection pressures–for example, amodiaquine and lumefantrine are intentionally combined because their complementary modes of action help minimise the emergence of resistance.[34] Notably, polymorphisms in the *pfcrt* and *pfmdr1* genes have been associated with differential susceptibility to these drugs, influencing treatment outcomes and the selection of resistant strains.[88]

Randomised clinical trials in Asia involving dihydroartemisinin-piperaquine-mefloquine and artemether-lumefantrine-amodiaquine have demonstrated that these combinations are well tolerated, safe and effective, even in regions with multidrug-resistant *P. falciparum* malaria.[89,90] In addition to these combinations, the ASAAP consortium is currently assessing the efficacy of the TACT artesunate-amodiaquine in combination with atovaquone-proguanil in children in Africa.[91] Two independent individual-based models of *P. falciparum* epidemiology and evolution were used to assess whether the introduction of either the TACT artesunate-mefloquine-piperaquine or artemether-lumefantrine-amodiaquine would reduce long-term artemisinin resistance levels and treatment failure rates in comparison to continued single partner drug ACT use. The results indicate that the introduction of TACTs could delay the spread of partner-drug resistance, thereby prolonging the efficacy of current antimalarial drugs.[92]

In **Chapter 4**, we assessed for the first time the effect of primaquine with the TACT artemether-lumefantrine-amodiaquine on gametocyte densities and transmission using mosquito feeding assays. We demonstrated that both artemether-lumefantrine alone and in combination with amodiaquine significantly reduce transmission by day 2 after treatment. The addition of a single-low dose of primaquine slightly enhanced this transmission-reducing effect by more rapidly clearing gametocytes and possibly achieving slightly faster gametocyte sterilisation. Gametocyte densities were minimally different between the artemether-lumefantrine and artemether-lumefantrine-amodiaquine combinations. However, in the group receiving the single low-dose of primaquine, we observed nearly complete gametocyte clearance by day 7 which was faster than for the non-primaquine treatments. These findings align with the data from **Chapter 3A**, indicating that artemether-lumefantrine has potent gametocyte-clearing and transmission-blocking effects and that primaquine may accelerate gametocyte clearance.

Artesunate-amodiaquine is used as the first-line ACT for uncomplicated *P. falciparum* malaria in many countries and is increasingly considered in areas where artemether-lumefantrine is showing reduced activity such as in parts of East Africa.[2] Importantly, the efficacy of artesunate-amodiaquine in reducing transmission has not been directly assessed. Findings on microscopically detected gametocyte carriage post-treatment suggest that it may not be very efficacious in reducing transmission potential.[93] While studies have shown persistent gametocyte carriage for 21 days or more after artesunate-amodiaquine treatment,[93-95] without data from transmission assays the infectivity of these gametocytes remains unclear. In **Chapter 4**, the effects of artesunate-amodiaquine, with and without primaquine, on gametocyte densities and transmission were evaluated. We revealed that gametocyte carriage persisted in all participants treated with artesunate-amodiaquine through day 28 of follow-up. Furthermore, three of 19 participants (three of 17 who were infectious at baseline) remained infectious to mosquitoes 14 days after treatment, with one participant still infectious at day 28 (end of follow-up). This finding confirms that artesunate-amodiaquine is an ACT with more limited transmission-blocking effects. The addition of transmission-blocking compounds such as primaquine or tafenoquine may be considered when artesunate-amodiaquine is deployed in areas of lumefantrine resistance.

In **Chapter 4**, one participant was found to be infectious on day 2, but not at baseline, while another was infectious on day 7, but not at baseline or day 2. In **Chapter 3**, there were similar instances where one participant was infectious on day 5, but not on day 2 or baseline, and another who was infectious at baseline and on day 5, but not on day 2. This suggests *de novo* generation of infectious gametocytes during or after treatment. However, since transmission to mosquitoes involves an inherent stochastic element, the absence of infected mosquitoes at a given timepoint does not necessarily rule out low levels of infectivity. Moreover, infectivity in these instances comes too early to be explained by newly developed gametocytes that take 10-12 days to mature.[96] This pattern may therefore also reflect differences in drug susceptibility or exposure of early gametocytes that could be released from sequestration in the bone marrow or spleen following initiation of treatment.

The trial discussed in **Chapter 4** was not designed to assess the clinical efficacy of TACT, nor did the study site record any partial artemisinin resistance at the time of the study. Future steps could involve testing TACT in clinical trials in African regions with partial artemisinin resistance to also assess its

transmission-reducing efficacy and compare it with the effectiveness of the ACTs currently used there.

The role of antimalarials in reducing transmission: concluding remarks and future perspectives

In conclusion, while ACTs remain the cornerstone of malaria treatment by rapidly reducing the asexual parasite load and clinical morbidity, their ability to reduce transmission differs among regimens. For example, artemether-lumefantrine has demonstrated potent transmission-reducing effects, whereas dihydroartemisinin-piperaquine and amodiaquine-artesunate show a more modest impact in the first month after treatment. Future strategies should focus not only on optimising the curative efficacy of antimalarials, but also on integrating additional gametocytocides. This includes incorporating low-dose primaquine or exploring the potential of the long-acting tafenoquine, particularly in areas with low transmission or where partial artemisinin resistance is emerging. SMC strategies may also be refined by including a gametocytocidal drug and expanding the target population to school-aged children to help reduce the infectious reservoir. Additionally, the emergence of partial artemisinin resistance underscores the importance of adaptive strategies, including refining combination regimens while addressing challenges in real-world implementation, such as drug stock-outs, regimen complexity, and patient adherence. Our findings from Mali reveal robust transmission-reducing activity of several antimalarials. However, given that transmission dynamics and the effectiveness of antimalarials may change over time, continuous surveillance is important to ensure timely adjustments to intervention strategies. Ultimately, combining effective treatment regimens with targeted transmission-blocking measures and broader population coverage could support sustained progress toward malaria control.

P. falciparum transmission-blocking vaccines

Another approach to reducing malaria transmission is through immunisation that aims to induce the production of antibodies that target the sexual stages of the malaria parasite. When mosquitoes ingest a blood meal from these vaccinated individuals, the antibodies present in the blood can disrupt the development of the sexual stages of the parasite within the mosquito, thereby

preventing subsequent human infections. Transmission-blocking vaccine candidate antigens are expressed at various stages of the parasite life cycle: during the gametocyte and gamete stages (e.g. the proteins Pfs48/45 and Pfs230), the zygote and ookinete stages (Pfs25 and Pfs28), or on the mosquito midgut (AnAPN1).[97] Naturally acquired immune responses against antigens expressed on the surface of mature gametocytes develop in individuals living in malaria-endemic areas. Evidence suggests that such anti-gametocyte immunity can block parasite development in the mosquito, thereby playing a role in transmission reduction.[97]

The standard membrane feeding assay (SMFA) is considered one of the "gold standard" assays for transmission-blocking vaccine development. In this assay, in vitro cultured *P. falciparum* gametocytes are fed through membrane feeders to a laboratory-reared colony of uninfected *Anopheles* mosquitoes.[98] Sera from participants who received a transmission-blocking vaccine or monoclonal antibody can be added to the gametocyte culture before feeding, allowing assessment of the transmission-reducing effect of the antibodies contained in the sera. Two main readouts have been used widely: transmission-reducing activity (TRA) and transmission-blocking activity (TBA). TRA refers to the reduction in oocyst count per mosquito, while TBA refers to the reduction in the prevalence of infected mosquitoes. Essentially, TRA reflects lowering the parasite load in mosquitoes, whereas TBA reflects the complete prevention of infection of individual mosquitoes. In reality, TRA and TBA go hand in hand and interventions that exert potent TRA (i.e. reduce oocyst numbers) will also reduce the number of mosquitoes with at least one oocyst (and thus have high levels of TBA).[99] Given the variability and lower precision associated with measuring low levels of TRA, a TRA of more than 80% is commonly defined as the efficacy threshold of interest.[97,99] This 80% TRA threshold helps standardise assessments and determine whether a vaccine candidate shows sufficient transmission-blocking potential to warrant further development, while recognising that lower efficacies may still have epidemiological relevance.[99]

Pfs25-based vaccines have shown potential in inducing antibodies that target *P. falciparum* sexual stage antigens, but they have not yet achieved sufficient or long-lasting TRA in humans. Various formulations, including the Pfs25-EPA/Alhydrogel® vaccine, recombinant plant-expressed Pfs25 VLP-FhCMB, and ChAd63/MVA vectors encoding Pfs25-IMX313, have demonstrated safety and immunogenicity with antibodies detected in most vaccinated participants.[100-103]

However, the antibody levels typically decline quickly, returning to near baseline within a year. TRA, as measured by SMFA, was generally low after the initial doses, though it increased in some participants following a booster dose. Despite this, only a few participants achieved even >50% TRA, and TRA estimates waned quickly over time. The weak and transient TRA suggests that improved formulations and probably alternative antigens are necessary to achieve sustained TRA.

An alternative antigen for transmission-blocking vaccines is Pfs230, an antigen known to be the target of both naturally occurring and vaccine-induced transmission-blocking antibodies.[96,104] Pfs230-based vaccines may thus benefit from natural boosting during natural *P. falciparum* infections, or, conversely, may enhance pre-existing low-level immunity by stimulating Pfs230-specific memory B cells generated through prior exposure. The Pfs230D1 vaccine has demonstrated its potential by inducing durable and functional antibody responses that target the gametocyte surface, effectively reducing parasite transmission to mosquitoes. In malaria-naive participants from the United States, TRA above 90% was observed in two out of five individuals from the Pfs230D1-EPA group and in one out of five individuals in the Pfs25+Pfs230D1 combination group, at two weeks following the second vaccine dose.[105] In Mali, participants receiving Pfs230D1 exhibited >80% TRA two weeks after the fourth vaccination, with a peak TRA of 89% (range: 82-96%).[106] In a Pfs25+Pfs230D1 combination group, a peak TRA of 85% (range 78-92%) was observed two weeks after the fourth vaccination.[106] TRA persisted at 74% (range: 64-83%) for 10 weeks after the fourth vaccination with Pfs230D1.[106] In contrast, participants receiving Pfs25 alone did not achieve >80% TRA at any point during the study.

Pfs48/45-based transmission-blocking vaccine

Another promising candidate for transmission-blocking vaccine is the male gametocyte surface protein Pfs48/45, that plays an important role in the development of the parasite in the mosquito midgut. Antibodies targeting Pfs48/45 can effectively prevent transmission when ingested with the parasite during a bloodmeal.[107] In **Chapter 5**, we evaluated a Pfs48/45-based transmission-blocking vaccine, R0.6C, adjuvanted with aluminium hydroxide (AlOH) alone or in combination with Matrix-M, in a first-in-human trial with malaria-naive adults. The vaccine consists of the R0.6C fusion protein, a chimera consisting of the 6-cysteine C-terminal fragment of Pfs48/45 (6C) coupled to the N-terminal region of the asexual stage *P. falciparum*

Glutamate Rich Protein GLURP (R0) produced in *Lactococcus lactis*.[108-110] We demonstrated that four vaccinations with either 30 µg or 100 µg R0.6C was safe, but with a higher local reactogenicity in the Matrix-M groups after the second, third, and fourth vaccination. Anti-R0.6C and anti-6C IgG titres were measured by enzyme-linked immunosorbent assay (ELISA). We showed similar anti-R0.6C and anti-6C titres between the 30 µg and 100 µg R0.6C arms, but titres were higher in the arms receiving the vaccination adjuvanted with both AlOH and Matrix-M. IgG responses against 6C as a fraction of total IgG responses against R0.6C were 10% in sera from participants immunised with R0.6C/AlOH + Matrix-M and significantly higher, namely 40%, in participants immunised with R0.6C/AlOH alone. While R0.6C induced antibodies against the 6C fragment of Pfs48/45 in malaria-naive humans, their serum antibody levels were insufficient to achieve significant TRA when tested at physiological concentrations. The detection of low concentrations of anti-6C IgG responses in post-immunisation sera prompted additional SMFAs with concentrated IgG from samples collected 14 days after the fourth immunisation. These experiments were performed with samples from six participants with the highest responses. The purified and concentrated R0.6C-specific antibodies, tested in two independent SMFA experiments, showed estimated TRAs ranging from 22% to 99%. This shows that R0.6C induces functional Pfs48/45-specific transmission-blocking antibodies, albeit at insufficient serum concentrations to result in transmission reduction at physiological concentrations. Current findings therefore highlight that 6C-based vaccines may be viable for future malaria transmission-blocking strategies but require further development, including more potent vaccine formulations and possibly the use of multi-component vaccines, to enhance immunogenicity.

Different Pfs48/45-based transmission-blocking vaccine formulations

Multiple strategies involving Pfs48/45 are currently under evaluation. The R0.6C vaccine tested in **Chapter 5** used one of the three domains of Pfs48/45, the C-terminal domain, as a vaccine immunogen, as this domain contains the epitope for the most potent known transmission-blocking antibody, 85RF45.1.[111] However, the structure of Pfs48/45 reveals that all three of its domains are exposed to the immune system. Evidence supporting the efficacy of antibodies targeting regions of Pfs48/45 beyond the C-terminal domain suggest that these are also effective at blocking transmission.[107] This approach is currently being explored in the United Kingdom with a full-length Pfs48/45 in malaria-naive adults (NCT05400746).

As observed in **Chapter 5**, the majority of the total IgG response against R0.6C in our vaccinated participants targets the GLURP R0 domain of this recombinant sub-unit vaccine, which is mainly expressed in asexual blood stage parasites.[112] This R0 fragment was included in the R0.6C vaccine construct in order to conformationally stabilise the 6-cystein (6C) fragment of the C-terminal domain of Pfs48/45, but in itself induces no sexual-stage antibodies. Replacing the R0 domain with another 6C-stabilising component that is either less immunodominant, or that itself also induces transmission-blocking antibodies, could be a more beneficial strategy. One promising candidate is the Pro domain of Pfs230. This is currently being explored with the multistage malaria vaccine candidate ProC6C.[96] The ProC6C vaccine includes the Pro domain of Pfs230, the 6C domain of Pfs48/45, and a short *P. falciparum* circumsporozoite protein (CSP) sequence.[113] In a phase 1 trial in Burkina Faso, the ProC6C/AlOH with and without Matrix-M and the R0.6C/AlOH with and without Matrix-M were administered to healthy adults.[114] The results showed that ProC6C/AlOH with Matrix-M achieved functional TRA in participants, with 13 out of 20 (65%) participants who received 100 µg ProC6C-AlOH with Matrix-M showing greater than 80% TRA. In contrast, R0.6C vaccination did not result in higher TRA compared to the control group.[114] An interim analysis of a subsequent phase 1 clinical trial in Mali with Pfs230, R0.6C, and ProC6C vaccine candidates demonstrated similar findings (ISRCTN13649456).[114]

Another consideration for optimising the immunogenicity of R0.6C is changing and optimising the vaccine delivery platform. Virus-like-particles (VLPs) and mRNA vaccines represent promising alternatives to the approach we used in **Chapter 5**. One study explored the use of VLPs to enhance the immunogenicity of a malaria transmission-blocking vaccine based on the Pfs48/45 antigen. The SpyTag/SpyCatcher system was used to covalently attach recombinant Pfs48/45 proteins (R0.6C and 6C) to Acinetobacter phage AP205 VLPs, creating highly ordered antigen displays.[115] The VLP-conjugated vaccines were tested in mice, revealing that VLP-display significantly increased antigen-specific IgG responses, particularly when formulated with the Montanide ISA 720 VG adjuvant. Functional assays showed that antibodies induced by the R0.6C-VLP vaccine had stronger TRA compared to soluble antigen formulations, supporting the potential of VLP-based malaria vaccines for clinical development.

Given the success of mRNA-based vaccines in recent years, their application to transmission-blocking vaccines may offer new opportunities to advance the

field. One study evaluated the potential of mRNA vaccines encoding the malaria transmission-blocking antigens Pfs25 and Pfs230D1.[116] Different mRNA constructs were tested in mice and compared to protein-based vaccines, the mRNA vaccines inducing stronger TRA in the SMFA. The immune response was durable, with >99% TRA sustained for 126 days following immunisation with the Pfs25 mRNA vaccine with a GPI anchor or TMI domain.[116] These findings show the potential of VLP and mRNA-based transmission-blocking vaccines, but further optimisation and clinical studies are required to confirm their potential in achieving stronger and more durable TRA in humans.[117]

Novel tool against *P. falciparum*: monoclonal antibodies

One of challenges with the R0.6C vaccine observed in **Chapter 5**, as well as with other transmission-blocking vaccines, is achieving sufficiently high and durable antibody levels to achieve a period of effective TRA. In addition to optimising vaccine components and enhancing formulations with adjuvants, it is also possible to directly administer a Pfs48/45-based monoclonal antibody. This approach could deliver rapid transmission-reduction and bypasses the need for vaccinees to generate immune responses. Monoclonal antibody strategies are increasingly considered as a potent tool to support malaria control and/or elimination efforts and may target various stages of the malaria parasite's lifecycle.

Several monoclonal antibodies aimed at the prevention of malaria infection are undergoing phase 1 and 2 clinical trials. These include CIS43LS and L9LS, two human monoclonal antibodies that target a highly conserved epitope on the circumsporozoite protein of *P. falciparum*.[118-120] CIS43LS and L9LS prevent malaria by neutralising sporozoites.[120,121] The sporozoites are inhibited from entering hepatocytes, the first stage of the parasite's lifecycle in the human host, thereby preventing both the development of the pathogenic blood stage and onward transmission of the parasite.

Transmission-reducing monoclonal antibody TB31F

The most advanced transmission-reducing monoclonal antibody candidate is TB31F, a humanised form of the rat monoclonal antibody 85RF45.1.[111] In **Chapter 6**, we evaluated TB31F for the first time in malaria naive adults in the Netherlands. Participants received a single intravenous dose of 0.1, 1, 3, or 10 mg/kg TB31F or a subcutaneous dose of 100 mg TB31F and were monitored

for three months. Both intravenous and subcutaneous administration of TB31F were well tolerated. When administered intravenously at doses of 0.1, 1, 3, and 10 mg/kg, TB31F resulted in geometric mean maximum concentrations (Cmax) of 2.94 µg/mL (95% CI 2.34–3.69), 28.3 µg/mL (14.4–55.5), 80.8 µg/mL (55.2–118), and 255 µg/mL (177–367), respectively, with peak concentrations occurring at the end of infusion (Tmax). Subcutaneous administration of 100 mg TB31F yielded a Cmax of 9.28 µg/mL (95% CI 3.49–24.7), occurring at a median of 4.3 days, with a bioavailability of 52%. The terminal half-life of TB31F was estimated to be 32.2 days. The functional activity of TB31F in participants' serum was assessed in SMFA. A single intravenous dose of 1 mg/kg, 3 mg/kg, or 10 mg/kg maintained >80% TRA against the West African *P. falciparum* strain NF54 for at least 28, 56, and 84 days, respectively, in all participants. Additionally, a single intravenous dose of 10 mg/kg sustained >80% TRA against the genetically distant Asian *P. falciparum* isolate NF135 for at least 84 days. Following subcutaneous administration of 100 mg TB31F, >80% TRA was sustained for at least 28 days in four of five participants. We demonstrated that a single intravenous administration of up to 10 mg/kg TB31F had a pharmacological profile suggesting TRA of >80% is maintained for 4-5 months.

In **Chapter 6**, passive immunisation with TB31F achieved robust TRA and demonstrated that a concentration of 2.1 µg/mL would be needed to achieve >80% TRA (IC80). This contrasts with the minimal TRA observed in participants immunised with Pfs48/45-based vaccine R0.6C/AlOH with Matrix-M in **Chapter 5**. Although the geometric mean anti-6C IgG concentration was approximately 5.4 µg/mL, functional TRA was not achieved. This could be explained by the fact that the measured anti-6C IgG concentration following R0.6C vaccination reflects the total binding antibody pool, comprising antibodies with varying affinities and specificities,[107,122] and does not directly indicate functional potency. In contrast, TB31F is a well-characterised monoclonal antibody optimised for high-affinity binding to a specific transmission-blocking epitope on the 6C domain. In the vaccine context, only a subset of the anti-6C antibodies appear to be functionally active. Factors such as lower affinity and suboptimal epitope targeting may reduce the effective concentration of transmission-blocking antibodies.

TB31F presents a promising alternative or complement to 8-aminoquinolines in *P. falciparum* transmission-blocking strategies. While primaquine and tafenoquine are effective gametocytocidal drugs, the extended duration

of activity of TB31F is advantageous in preventing the transmission of gametocytes that sequester in the bone marrow and enter circulation at later stages, potentially evading the effects of the gametocytocidal drugs. When combined with antimalarial drugs targeting asexual blood stages, TB31F could form part of a comprehensive single-encounter therapy.

The half-life of monoclonal antibodies can be extended through several strategies, including modifications to the Fc region to increase binding affinity to the neonatal Fc receptor, which can result in a two to four times extension of the half life.[123-125] In the case of the preventive malaria monoclonal antibodies CIS43LS and L9LS, half-life extension was performed prior to human trials. In rhesus macaques, the half-life of CIS43LS was increased from 22 days to 39 days compared to the parent antibody CIS43.[126] In the first-in-human trial of CIS43LS, the half-life was determined to be 56 days,[127] reflecting a 2.5-fold increase over the initial half-life in rhesus macaques. If the half-life of TB31F were to be extended two to four times, it is conceivable that a single 10 mg/kg dose could maintain >80% TRA for approximately 1 to 2 years, respectively. Extending the half-life of TB31F can reduce dosing frequency, lower the required administration volume, and improve cost-effectiveness, thereby facilitating subcutaneous delivery for mass distribution in malaria-endemic areas. However, because monoclonal antibodies do not induce durable immunological memory in populations with ongoing exposure, repeated administration remains necessary. Extending the half-life of TB31F may help reduce the frequency of dosing by ensuring sufficient transmission-reducing activity to cover an entire transmission season, but it is unlikely to eliminate the need for re-administration altogether.

While intravenous administration, as used in **Chapter 6**, was suitable for the first-in-human trial, subcutaneous administration should be considered for future applications. Subcutaneous administration is easier to implement, more comfortable for patients, reduces administration costs, and minimises resource use. However, subcutaneous delivery is limited by the maximum volume that can be administered. As proof of concept, TB31F was also administered subcutaneously to five participants in **Chapter 6**. Since subcutaneous injections are typically restricted to 1-2 mL, it makes it more challenging to achieve the higher doses that can be reached with intravenous administration. To overcome this limitation, increasing the concentration is an option, but the current TB31F formulation (50 mg/mL) is considered to already be at the upper limit for subcutaneous administration. Alternative strategies

include multiple subcutaneous injections, though this may be less feasible in paediatric populations, or co-administration with hyaluronic acid. The subcutaneous matrix of hyaluronic acid and collagen fibers restricts injection volumes, but enzymatic degradation of hyaluronic acid using recombinant human hyaluronidase temporarily increases dispersion capacity, allowing for larger fluid volumes.[128] Whilst this offers opportunities to increase the administered dose in some circumstances, it is questionable whether this is an operationally attractive strategy for widescale implementation in low-resource settings.

In **Chapter 6**, the efficacy of the monoclonal antibody TB31F was assessed by SMFA, with *Anopheles stephensi* mosquitoes and cultured *P. falciparum* gametocytes from the west African NF54 strain and the genetically distant Asian NF135 strain. The SMFA is considered the gold standard for assessing the efficacy of transmission-blocking interventions,[97] as it involves high numbers of gametocytes and oocysts from a single strain, making it an effective tool for reproducibly measuring the relative efficacy of transmission-reducing interventions in early clinical studies. Nonetheless, it does not exactly replicate natural transmission conditions, but is a more stringent measure of efficacy against a limited number of *P. falciparum* strains. In addition to SMFA, two field-based mosquito feeding assays-direct skin feeding (DSF) and direct membrane feeding assay (DMFA)-are commonly used to assess infectiousness of *P. falciparum* in naturally infected individuals. DSF closely mimics natural transmission by allowing mosquitoes to feed directly on the skin of infected individuals, generally resulting in higher proportion of infected mosquitoes.[65] In DMFA, venous blood is collected from naturally infected individuals and offered to mosquitoes via membrane feeding. This allows for larger mosquito numbers, gametocyte quantification, the use of naturally circulating parasite strains, and locally caught and reared mosquitoes. To assess the efficacy of TB31F against naturally acquired gametocytes in conditions closer to natural human-to-mosquito transmission, subsequent studies could incorporate DMFA and DSF alongside SMFA.[129,130] While SMFA remains the gold standard for the assessment of TRA, DMFA and DSF provide complementary insights by using blood from naturally infected individuals and locally relevant mosquito species. Notably, research has demonstrated that the effectiveness of certain transmission-blocking antibodies, such as the anti-Pfs25 monoclonal antibody 4B7, can vary between mosquito species like *A. stephensi* and *A. gambiae*,[131] underscoring the importance of evaluating interventions across different

vectors. Integrating these assays can offer a comprehensive understanding of TB31F's potential impact in diverse epidemiological settings.

Potential use-scenarios of TB31F

While most research conducted so far has focused on monoclonal antibodies aimed at preventing malaria infection, TB31F opens up several avenues for interrupting malaria transmission. One potential strategy involves the standalone deployment of TB31F across all age groups at risk of *P. falciparum* infection and contributing to transmission. In this scenario, TB31F could be integrated into existing public health programs—such as routine immunisation or seasonal malaria chemoprophylaxis campaigns—to complement vector control measures. By targeting a broad segment of the population, standalone administration with a transmission-reducing monoclonal antibody could substantially lower community-level transmission, particularly in settings where conventional interventions have reached a plateau in efficacy or where residual transmission persists despite control efforts.

A second strategy under consideration is the co-administration of TB31F with an antimalarial suitable for mass drug administration (MDA). MDA campaigns aim to treat the entire population and thereby reducing the parasite reservoir in the community. Currently, dihydroartemisinin-piperaquine is most commonly used for MDA due to piperaquine's long elimination half-life, resulting in 4-6 weeks of post-treatment prophylaxis. A study in a low-to-moderate transmission setting in Senegal compared the impact of three cycles of MDA with dihydroartemisinin-piperaquine plus a single low dose of primaquine to four cycles of SMC with sulfadoxine-pyrimethamine plus amodiaquine.[132] While MDA plus a single low dose of primaquine led to a reduction in malaria burden during the intervention year, its long-term impact was limited,[132] requiring another transmission-reducing strategy or continuation of MDA. Given the short half-life of primaquine, TB31F could potentially offer a more durable interruption of *P. falciparum* transmission, whilst also contributing to artemisinin resistance containment and serving as an alternative to 8-aminoquinolines for transmission reduction.

A third scenario involves combining TB31F with a monoclonal antibody that targets CSP, such as CIS43LS or L9LS. This dual-antibody approach offers two advantages over the use of a single infection-preventing monoclonal antibody: it provides direct protection to individuals while also helping to reduce the spread of *P. falciparum* strains resistant to CIS43LS/L9LS. By

preventing the uptake and transmission of escape mutants by mosquitoes, this combination could address a potential vulnerability of these monoclonal antibodies–their long half-life, which may result in prolonged subinhibitory serum concentrations that could favour the selection of parasites with reduced binding affinity. TB31F would thus not aim to reduce community-wide transmission (as in some other intervention scenarios) but would be used to protect an infection-preventing monoclonal antibody.

Community-level impact of TB31F

Challenges may be anticipated when aiming to include the entire community, especially in large urban settings where logistical and cost constraints may prevent achieving the high coverage required for maximal impact. To address this, it is necessary to understand which subgroups contribute most to the infectious reservoir and their relative importance in sustaining transmission. Previous work demonstrated that school-aged children are the key infectious reservoir in many endemic areas.[73] Repeated exposure induces a state of partial immunity in this age group, that often results in asymptomatic or subclinical infections that remain untreated, allowing gametocytes to persist for extended periods.[72,133,134] Furthermore, epidemiological studies suggest that behavioural and physiological factors, such as increased outdoor activity, inconsistent use of vector control measures, and a larger body surface area, contribute to a higher frequency of mosquito bites in school-aged children compared to younger children.[135] The interplay of these immunological and behavioural determinants not only prolongs the period of infectivity but also amplifies the role of school-aged children in maintaining malaria transmission within endemic regions. Transmission blocking interventions that fail to include this population may therefor have a reduced community impact.

Simulation of the community-level impact of demographically targeted TB31F administration in areas with seasonal malaria transmission, amid ongoing malaria control efforts, suggest that TB31F would be effective in reducing the human infectious reservoir and preventing clinical malaria episodes within the community.[136] Modeling suggests that in a high-transmission setting (**Figure 3**), malaria prevalence and clinical incidence decrease markedly when TB31F is introduced, particularly when combined with SMC using sulfadoxine-pyrimethamine plus amodiaquine.

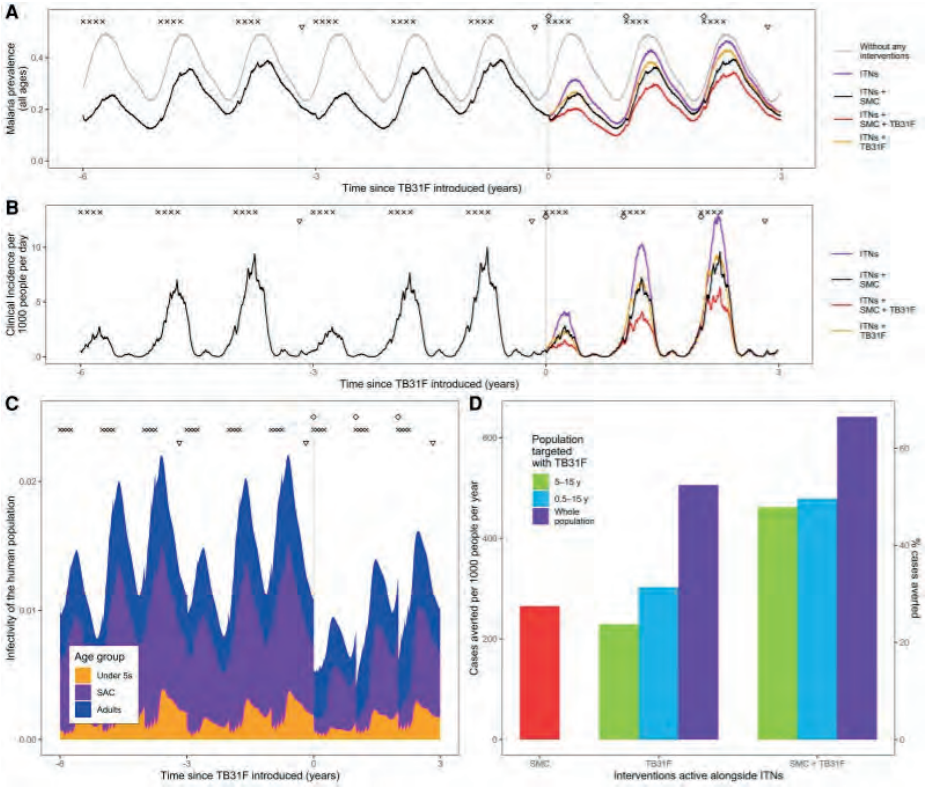

Figure 3. Modelling the introduction of TB31F intervention in the Sahel region of Burkina Faso (high-transmission setting). *A*, Malaria parasite prevalence (by microscopy) and changes over time as interventions additional to insecticide-treated nets (ITNs) and seasonal malaria chemoprevention (SMC) are introduced. The symbols at the top of the plot indicate the timings of intervention delivery. We assume that ITN distribution campaigns (triangles) and SMC delivery (crosses) have occurred regularly prior to the introduction of TB31F (diamonds) at time zero. The grey curve indicates malaria prevalence prior to the introduction of control interventions. The coloured lines indicate the interventions used after time zero. SMC is delivered to children aged 3–59 months at 80% coverage; in *A*–*C*, TB31F is delivered to school-aged children (SAC) (5–15 years of age) at 80% coverage. *B*, Model-derived estimates of clinical incidence in the population over time. The impact of TB31F is most apparent in the second and third years of rollout, as the new ITNs prevent a high proportion of cases in the first year. *C*, The changing infectivity of the human population to a blood-feeding mosquito as interventions are introduced. This panel shows how the infectious reservoir (not adjusted for age-dependent mosquito biting rates) of malaria is distributed among 3 age groups: children <5 years of age, SAC, and adults (>15 years of age). After TB31F is introduced to SAC, the infectiousness of this age group is greatly reduced for several months. *D*, Cases averted due to SMC and TB31F interventions. Over a 3-year period, we measure the reduction in cases on top of those prevented by ITNs. Here we vary the age group targeted with TB31F (SAC, all children aged >6 months, and all age groups aged >6 months in the community), assuming a coverage of 80% within the target age group. We quantify the clinical cases averted in 2 ways: the number of cases averted per 1000 people per year (left y-axis) and the percentage of the total number cases that is averted (right y-axis). In terms of cases averted per dose of TB31F administered, targeting children aged 5–15 years proved more efficient than targeting children aged 0.5–15 years, or the whole

population aged >6 months. *Reproduced from Challenger JD, van Beek SW, Ter Heine R, et al. Modeling the Impact of a Highly Potent Plasmodium falciparum Transmission-Blocking Monoclonal Antibody in Areas of Seasonal Malaria Transmission. J Infect Dis 2023; 228(2): 212-23. Author rights retained under publisher policy for reuse in academic theses.*

We also simulated what population would allow for the most efficient intervention, identifying that focusing on subgroups with the highest contribution to transmission yields greater per-dose effectiveness. While the impact would be maximum if all demographic populations that contribute to transmission are included in administration campaigns, targeting school-aged children (5-15 years) proves to be the most efficient strategy in terms of cases averted per dose administered. Over a three-year period, administering TB31F combined with SMC to this target population is predicted to avert 48% of all clinical cases (462 cases per 1,000 people per year), while expanding coverage to the entire population increases the reduction to 66% (641 cases per 1,000 people per year). In the low-transmission setting (**Figure 4**), modeling suggests that the impact of TB31F is even more pronounced, with a 53% reduction in clinical cases (141 cases per 1,000 people per year) when targeting school-aged children and an 82% reduction (217 cases per 1,000 people per year) when administered to the entire population. These findings support the potential of annual administration of the monoclonal antibody TB31F as an effective strategy to reduce malaria transmission and disease burden in areas with seasonal malaria transmission.

TB31F follow-up study in malaria-exposed individuals

The results of our phase 1 trial in **Chapter 6** supported the progression of TB31F to a phase 1/2a trial that is currently underway in Mali (NCT06413108). This study aims to establish TB31F safety and transmission-reducing activity in naturally infected individuals. It comprises two cohorts: a safety cohort and an efficacy cohort (**Figure 5**). The safety cohort enrols 75 health participants, randomised 2:1 to receive subcutaneous TB31F (10 mg, 100 mg, or 200 mg) or placebo. This cohort follows an dose-escalation and age de-escalation design to determine the safety and pharmacokinetic profile of TB31F in both adults and children. The efficacy cohort consists of 90 asymptomatic gametocyte carriers, also randomised 2:1 to receive TB31F (40 mg or 100 mg) or placebo. All efficacy cohort participants receive dihydroartemisinin-piperaquine at symptom onset or on day 14, with follow-up continuing until day 84 post-administration.

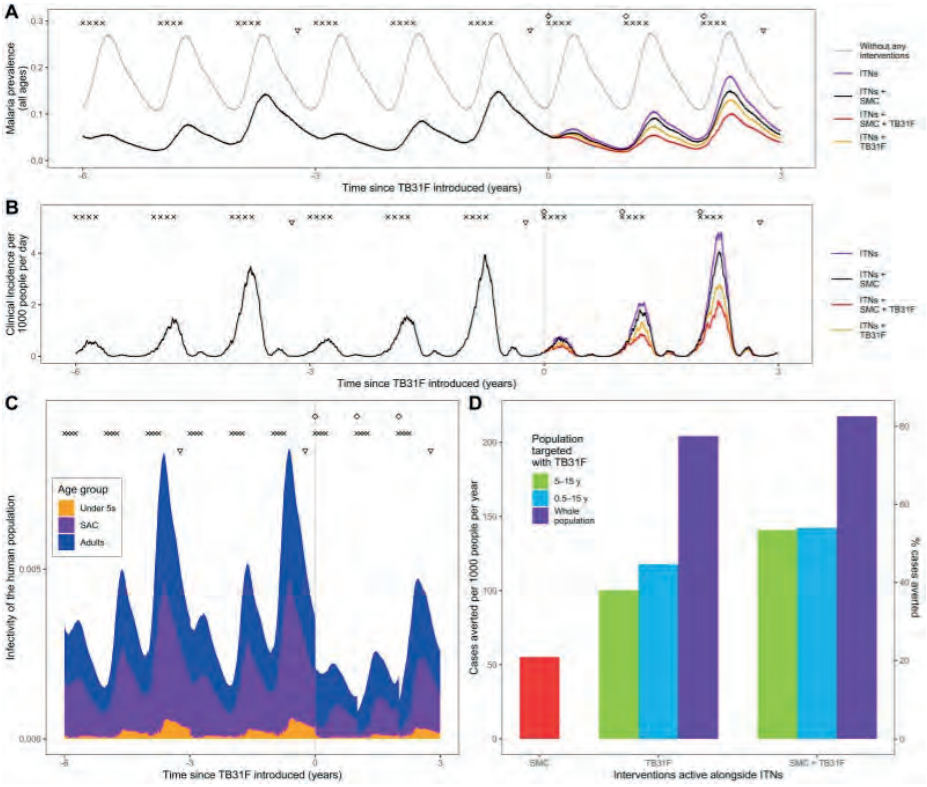

Figure 4. Modelling the introduction of TB31F intervention in the Upper River region of the Gambia (low-transmission setting). See legend of Figure 3 for details. *Reproduced from Challenger JD, van Beek SW, Ter Heine R, et al. Modeling the Impact of a Highly Potent Plasmodium falciparum Transmission-Blocking Monoclonal Antibody in Areas of Seasonal Malaria Transmission. J Infect Dis 2023; 228(2): 212-23. Author rights retained under publisher policy for reuse in academic theses.*

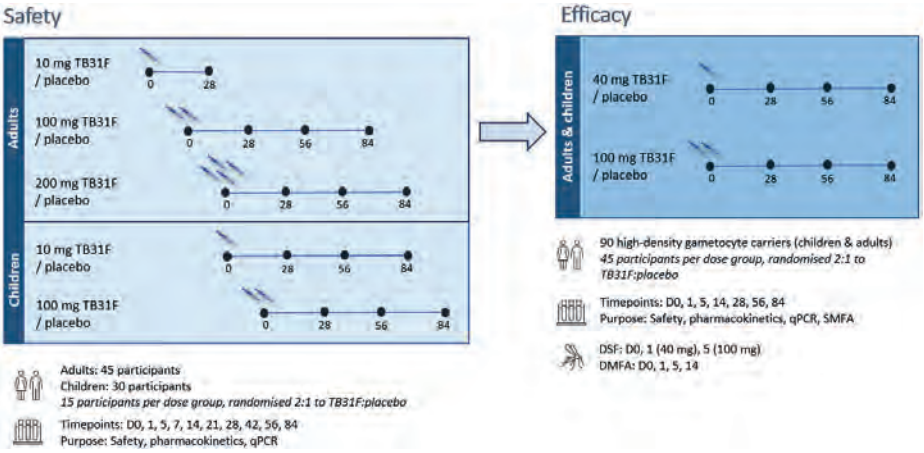

Figure 5. Phase 1/2a TB31F study design

While **Chapter 6** focused exclusively on adults, children are included in the current study as they are an important target population for this intervention. Monoclonal antibody use in children is considered feasible, as demonstrated by the successful deployment of palivizumab and nirsevimab for the prevention of respiratory syncytial virus in children.[137,138] In **Chapter 6**, TB31F was administered intravenously with pre-medication (1000 mg paracetamol and 2 mg clemastine). In contrast, in the current trial, TB31F is first administered without pre-medication in adults before including children. Given that intravenous doses of up to 920 mg TB31F were well tolerated in the phase 1 trial, a subcutaneous dose of up to 200 mg in the current study was considered feasible. However, without hyaluronidase, administering 200 mg subcutaneously would require four injections—a regimen that may be acceptable for adults but is less suitable for children.

For the efficacy cohort, only high-density gametocyte carriers (≥16 gametocytes/μL) are included, in line with our approach used in **Chapters 2, 3A, and 4**. This is the first study where our approach that was developed for the evaluation of antimalarial treatment, is expanded to evaluate immunological intervention. Efficacy will be assessed using three mosquito feeding assays: DSF, DMFA, and SMFA. This allows us to test TB31F against naturally acquired gametocyte isolates and locally relevant mosquitoes species through DSF and DMFA.

The anticipated outcomes of this trial are to demonstrate that TB31F doses up to 200 mg are safe and well tolerated in Malian adults, and that doses up to 100 mg are similarly safe in children aged ≥10 years. In addition, the trial aims to confirm that subcutaneous TB31F administration effectively prevents transmission of P. falciparum, as measured by reductions in mosquito infection rates in SMFA, DMFA, and DSF. Furthermore, it will assess whether a consistent relationship can be defined between TB31F serum concentrations and transmission-reducing activity across all three assays. It is expected that pharmacokinetic profiles in Malian participants will align with those previously observed in malaria-naïve adults in the Netherlands, thereby supporting the potential for a single-dose TB31F regimen to interrupt transmission over an entire malaria season.

In summary, these findings demonstrate that TB31F is a promising candidate for integration into existing malaria control strategies. Its long half-life, which could be extended further, and sustained transmission-reducing activity

suggests that a single annual administration of TB31F may be operationally feasible and could enhance the effectiveness of SMC. As malaria transmission decreases, targeted administration of TB31F to school-aged children may offer a strategy to accelerate progress toward malaria elimination. The potential of TB31F to complement current interventions and reduce transmission in both high- and low-endemicity settings underscores its potential as a novel tool in malaria control.

Navigating sustainability in malaria research

In a warming world, rising temperatures and changing weather patterns continue to affect the health, security, and livelihoods of people across the globe. Vulnerable communities in Africa are particularly affected by the most severe impacts of climate change, and many of these same communities are also at high risk of contracting malaria. Vector-borne diseases, driven by environmental and climatic factors, are sensitive to climate change.[139] However, it remains unclear whether malaria burden will increase in response to rising temperatures.[140] Over the past century, the global malaria burden has declined, despite a clear rise in mean temperatures.[80] At the same time, the geographical areas and number of months per year that conditions are favourable for malaria transmission are likely to increase in response to global warming.[80,140] This underscores the need for sustained intervention coverage and efficacy, as climate change may contribute to an expanded transmission window and range, posing an additional challenge to malaria control efforts.

Addressing climate change in clinical research requires focus on both adaptation and mitigation. Clinical trials generate CO_2-equivalent (CO_2e) emissions comparable to those of entire nations. For instance, the cumulative emissions from the approximately 350,000 clinical trials registered on ClinicalTrials.gov as of 2021 have been estimated at 27.5 million tons of CO_2e.[141] This is slightly more than 30% of Bangladesh's total annual emissions, a country with a population of 163 million. These emissions primarily stem from energy consumption at research facilities, transportation, and air travel. However, research on the environmental impact of clinical trials on the African continent remains limited and key emission drivers may differ from those in other regions. Moreover, studies may have different *hotspots* for interventions that allow researchers to optimize their practices to perform high-quality research at minimal environmental cost. To inform and support sustainable

research practices, we conducted a life cycle assessment (LCA) of the clinical malaria trial described in **Chapter 3A**. This analysis, presented in **Chapter 3B**, represents the first LCA of a clinical trial extending beyond carbon footprint analysis alone. Our analysis estimated that the environmental impact of the trial in **Chapter 3A** is approximately 20.5 metric tons of CO_2e emissions, with the largest contributors being international travel (50%), electricity consumption in Mali (28%), and the air transportation of materials (14%). The primary contributors to ecosystem damage were carbon emissions, terrestrial acidification, and ozone formation.

Implementing energy-efficient practices and integrating renewable energy sources are important steps in minimising emissions. Notably, international travel was identified as the largest contributor to CO_2e emissions, with trial members traveling 56,600 km to attend a conference, and by doing so, emitting approximately 10,200 kg of CO_2e emissions. This raises the question if it is still justifiable for over half of a study's emissions to stem from presenting results that are already accessible via preprint servers? Or is in-person dissemination essential to ensure the uptake and application of research findings? One option could be to prioritise virtual or hybrid conference formats, which have been shown to significantly reduce CO_2e emissions. For instance, transitioning from in-person to virtual conferencing can reduce the carbon footprint by 94%.[142] Many conferences now offer virtual attendance options or are fully virtual, like the Women in Malaria Conference, which held its first conference in 2021.[143] Furthermore, regional or decentralised conferences with multiple venues can further reduce the need for intercontinental travel, shorten travel distances, and make public transportation more feasible, substantially lowering CO_2e emissions.[144] Responsibility for implementing such changes lies not only with policymakers but also with funders and academics, who collectively influence the organisation and participation in academic conferences.

Adopting more sustainable practices often requires significant upfront investments, which may be challenging to secure from funding bodies that primarily focus on research activities. For instance, if solar panels would have been implemented at the malaria research unit in Ouélessébougou, this would have reduced the CO_2e emissions of the clinical trial described in **Chapter 3A** by 28%. At the Medical Research Council Unit in The Gambia, the London School of Hygiene and Tropical Medicine installed the largest array of solar panels in country.[145] This alternative power source supports their research practices and saves around 1,250 tonnes of CO_2e emissions per year.[145] A similar solar

power system at the malaria research center in Ouélessébougou could lower CO_2e emissions not just for a single trial, but for all ongoing and future studies conducted there. In addition to reducing fossil fuel dependency, this system would lower electricity and diesel costs while mitigating the impact of utility grid blackouts. However, the initial investment required for such an installation is large and it is unlikely that the financial savings alone would justify the investment in the short term. Advocating for this initiative would thus need to focus primarily on its long-term sustainability benefits, which can be a more challenging case to make to funding bodies.

The findings of **Chapter 3B** aim to inform researchers, institutions, and funding bodies, supporting the adoption of more sustainable practices and contributing to the development of environmental policies for future clinical trials. As part of these efforts, our trial data will be used to evaluate the guidance developed by the Greener Trials group.[146] This group has produced a comprehensive methodology for assessing the carbon footprint of clinical research, including detailed guidance documents, data collection tools, and a quick guide to support trialists in calculating and understanding the environmental impact of their studies.[147] Our trial will serve as a case study to apply this methodology and evaluate how the estimated emissions compare. By contributing our data, we can help expand and refine the guidance—improving its applicability to diverse clinical research settings, particularly those conducted in low-resource environments. This collaboration will also support the development of an open-science database and digital tools to automate carbon footprint calculations in clinical trials. Such tools can be used to identify carbon-intensive *hotspots* within trials and to inform targeted mitigation strategies. Ultimately, these resources will make calculating carbon footprint more accessible and streamlined for future users, and strengthen the evidence base for implementing greener clinical research practices.

Concluding remarks

The COVID-19 pandemic demonstrated how quickly societies can react to large-scale public health challenges. Measures such as lockdowns, restricted freedoms, and the adoption of preventive measures, including vaccinations, were primarily implemented to protect vulnerable populations and achieve herd immunity, often without immediate personal benefit. While these interventions initially held widespread public support, this support

diminished over successive waves. These lessons should not be ignored when preparing for other epidemic or endemic diseases. The transmission-reducing strategies discussed in this thesis—whether through antimalarials, vaccines, or monoclonal antibodies—aim to reduce malaria transmission at the community level. However, the extent to which individuals perceive a direct benefit from these interventions varies. Such "altruistic" interventions require strong public engagement and are more likely to succeed when cultural and societal contexts are taken into account. In some settings, community leaders may play a key role in promoting uptake. Identifying the right target groups and optimally timing interventions can improve effectiveness. It should also be noted that the traditional view on stakeholders and influential community members needs constant reviewing and updating. The African continent experiences both rapid urbanisation and the penetration of internet. Public health campaigns, especially those that hope for broad participation for community benefit, need to operate in a world where the influence of stakeholders is in constant flux.

Together, the studies presented in this thesis provide a comprehensive assessment of current and emerging strategies to interrupt P. falciparum transmission. The consistent application of mosquito feeding assays and harmonised methodologies has yielded robust insights into the transmission-reducing efficacy of antimalarials, gametocytocidal agents, transmission-blocking vaccine, and transmission-reducing monoclonal antibody. I have tried to highlight the importance of tailoring intervention strategies to local transmission dynamics and resistance patterns, while also showing the added value of integrating transmission-blocking tools into existing malaria control programmes. As the malaria landscape continues to evolve under pressure from biological, climatic, and operational challenges, reducing *P. falciparum* transmission further will require a combination of well-evaluated tools and strategies tailored to local contexts. Sustaining and advancing progress will require the strategic use of well-characterised antimalarial combinations, integration of transmission-blocking agents like primaquine or tafenoquine where appropriate, further evaluation of next-generation tools such as TB31F, and adaptation of interventions to local transmission patterns, resistance profiles, and societal developments.

References

1. World Health Organization. World malaria report 2023. Geneva, 2023.
2. World Health Organization. World malaria report 2024: addressing inequity in the global malaria response. Geneva, 2024.
3. World Health Organization. Malaria Threats Map. 2024. https://apps.who.int/malaria/maps/threats/ (accessed 26 december 2024).
4. Berhane A, Anderson K, Mihreteab S, et al. Major Threat to Malaria Control Programs by Plasmodium falciparum Lacking Histidine-Rich Protein 2, Eritrea. *Emerg Infect Dis* 2018; **24**(3): 462-70.
5. Ishengoma DS, Mandara CI, Bakari C, et al. Evidence of artemisinin partial resistance in northwestern Tanzania: clinical and molecular markers of resistance. *Lancet Infect Dis* 2024; **24**(11): 1225-33.
6. Asua V, Conrad MD, Aydemir O, et al. Changing Prevalence of Potential Mediators of Aminoquinoline, Antifolate, and Artemisinin Resistance Across Uganda. *J Infect Dis* 2021; **223**(6): 985-94.
7. Uwimana A, Legrand E, Stokes BH, et al. Author Correction: Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. *Nat Med* 2021; **27**(6): 1113-5.
8. Mihreteab S, Platon L, Berhane A, et al. Increasing Prevalence of Artemisinin-Resistant HRP2-Negative Malaria in Eritrea. *N Engl J Med* 2023; **389**(13): 1191-202.
9. Slater HC, Griffin JT, Ghani AC, Okell LC. Assessing the potential impact of artemisinin and partner drug resistance in sub-Saharan Africa. *Malar J* 2016; **15**: 10.
10. Ebong C, Sserwanga A, Namuganga JF, et al. Correction to: Efficacy and safety of artemether-lumefantrine and dihydroartemisinin-piperaquine for the treatment of uncomplicated Plasmodium falciparum malaria and prevalence of molecular markers associated with artemisinin and partner drug resistance in Uganda. *Malar J* 2022; **21**(1): 37.
11. Emiru T, Getachew D, Murphy M, et al. Evidence for a role of Anopheles stephensi in the spread of drug- and diagnosis-resistant malaria in Africa. *Nat Med* 2023; **29**(12): 3203-11.
12. Johnston GL, Smith DL, Fidock DA. Malaria's missing number: calculating the human component of R0 by a within-host mechanistic model of Plasmodium falciparum infection and transmission. *PLoS Comput Biol* 2013; **9**(4): e1003025.
13. Smith TS, A. Reproduction numbers in malaria and their implications. *Trends Parasitol* 2012; **28**(1): 3-8.
14. Klepac P, Hsieh JL, Ducker CL, et al. Climate change, malaria and neglected tropical diseases: a scoping review. *Trans R Soc Trop Med Hyg* 2024; **118**(9): 561-79.
15. IPCC. Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland, 2023.
16. Agency EM. EMA recommends first COVID-19 vaccine for authorisation in the EU. 2020.
17. Administration FaD. FDA Approves First COVID-19 Vaccine. 2021.
18. Fougeroux C, Goksoyr L, Idorn M, et al. Capsid-like particles decorated with the SARS-CoV-2 receptor-binding domain elicit strong virus neutralization activity. *Nat Commun* 2021; **12**(1): 324.

19. Tan CW, Lim BL, Young BE, et al. Comparative neutralisation profile of SARS-CoV-2 omicron subvariants BA.2.75 and BA.5. *Lancet Microbe* 2022; **3**(12): e898.
20. Mordmuller B, Sulyok M, Egger-Adam D, et al. First-in-human, Randomized, Double-blind Clinical Trial of Differentially Adjuvanted PAMVAC, A Vaccine Candidate to Prevent Pregnancy-associated Malaria. *Clin Infect Dis* 2019; **69**(9): 1509-16.
21. Esen M, Kremsner PG, Schleucher R, et al. Safety and immunogenicity of GMZ2 - a MSP3-GLURP fusion protein malaria vaccine candidate. *Vaccine* 2009; **27**(49): 6862-8.
22. Adjalley SH, Johnston GL, Li T, et al. Quantitative assessment of Plasmodium falciparum sexual development reveals potent transmission-blocking activity by methylene blue. *Proc Natl Acad Sci U S A* 2011; **108**(47): E1214-23.
23. World Health Organization. WHO Target Product Profiles for COVID-19 Vaccines. 2020. https://www.who.int/docs/default-source/blue-print/who-target-product-profiles-for-covid-19-vaccines.pdf.
24. Nordic B. Bavarian Nordic Reports 12-Month Durability Data from a Phase 2 Clinical Trial of its COVID-19 Vaccine Candidate. 2023.
25. Nordic B. Bavarian Nordic Reports Phase 3 Topline Results for its COVID-19 Booster Vaccine Candidate. 2023.
26. Nordic B. Bavarian Nordic Provides Update on COVID-19 Booster Vaccine Program. 2023.
27. Chen Q, Lai H. Plant-derived virus-like particles as vaccines. *Hum Vaccin Immunother* 2013; **9**(1): 26-49.
28. Bioways G. AdaptVac announces a collaboration with GTP Bioways to manufacture its cVLP based placental malaria vaccine. 2024.
29. Initiative EV. Placental Malarial Vaccines. https://www.euvaccine.eu/placental-malaria-vaccines (accessed 04-11-2024 2024).
30. Copenhagen Uo. Research on vaccines against potential future pandemics receives giant grant. 2023.
31. Kim S, Kang H, Skrip L, et al. Progress and challenges in Nipah vaccine development and licensure for epidemic preparedness and response. *Expert Rev Vaccines* 2025; **24**(1): 183-93.
32. WHO Global Malaria Programme. WHO Guidelines for malaria. Geneza, Switzerland, 2023.
33. Krishna S, Bustamante L, Haynes RK, Staines HM. Artemisinins: their growing importance in medicine. *Trends Pharmacol Sci* 2008; **29**(10): 520-7.
34. White N. Antimalarial drug resistance and combination chemotherapy. *Philos Trans R Soc Lond B Biol Sci* 1999; **354**(1384): 739-49.
35. White NJ, Ashley EA, Recht J, et al. Assessment of therapeutic responses to gametocytocidal drugs in Plasmodium falciparum malaria. *Malar J* 2014; **13**: 483.
36. Bousema T, Drakeley C. Epidemiology and infectivity of Plasmodium falciparum and Plasmodium vivax gametocytes in relation to malaria control and elimination. *Clin Microbiol Rev* 2011; **24**(2): 377-410.
37. Stepniewska K, Humphreys GS, Goncalves BP, et al. Efficacy of Single-Dose Primaquine With Artemisinin Combination Therapy on Plasmodium falciparum Gametocytes and Transmission: An Individual Patient Meta-Analysis. *J Infect Dis* 2022; **225**(7): 1215-26.
38. Bolscher JM, Koolen KM, van Gemert GJ, et al. A combination of new screening assays for prioritization of transmission-blocking antimalarials reveals distinct dynamics of marketed and experimental drugs. *J Antimicrob Chemother* 2015; **70**(5): 1357-66.

39. Lelievre J, Almela MJ, Lozano S, et al. Activity of clinically relevant antimalarial drugs on Plasmodium falciparum mature gametocytes in an ATP bioluminescence "transmission blocking" assay. *PLoS One* 2012; **7**(4): e35019.
40. van Pelt-Koops JC, Pett HE, Graumans W, et al. The spiroindolone drug candidate NITD609 potently inhibits gametocytogenesis and blocks Plasmodium falciparum transmission to anopheles mosquito vector. *Antimicrob Agents Chemother* 2012; **56**(7): 3544-8.
41. Schneider P, Bousema JT, Gouagna LC, et al. Submicroscopic Plasmodium falciparum gametocyte densities frequently result in mosquito infection. *Am J Trop Med Hyg* 2007; **76**(3): 470-4.
42. Butcher GA. Development of malaria blood-stage vaccines: learning from mosquitoes. *Trans R Soc Trop Med Hyg* 2007; **101**(6): 530-1.
43. Bradley J, Soumare HM, Mahamar A, et al. Transmission-blocking Effects of Primaquine and Methylene Blue Suggest Plasmodium falciparum Gametocyte Sterilization Rather Than Effects on Sex Ratio. *Clin Infect Dis* 2019; **69**(8): 1436-9.
44. Halsey ES, Plucinski MM. Out of Africa: Increasing reports of artemether-lumefantrine treatment failures of uncomplicated Plasmodium falciparum infection. *J Travel Med* 2023; **30**(8).
45. Sawa P, Shekalaghe SA, Drakeley CJ, et al. Malaria transmission after artemether-lumefantrine and dihydroartemisinin-piperaquine: a randomized trial. *J Infect Dis* 2013; **207**(11): 1637-45.
46. Bousema JT, Schneider P, Gouagna LC, et al. Moderate effect of artemisinin-based combination therapy on transmission of Plasmodium falciparum. *J Infect Dis* 2006; **193**(8): 1151-9.
47. Mwaiswelo RO, Ngasala B, Msolo D, Kweka E, Mmbando BP, Martensson A. A single low dose of primaquine is safe and sufficient to reduce transmission of Plasmodium falciparum gametocytes regardless of cytochrome P450 2D6 enzyme activity in Bagamoyo district, Tanzania. *Malar J* 2022; **21**(1): 84.
48. Sutherland CJ, Ord R, Dunyo S, et al. Reduction of malaria transmission to Anopheles mosquitoes with a six-dose regimen of co-artemether. *PLoS Med* 2005; **2**(4): e92.
49. Goncalves BP, Tiono AB, Ouedraogo A, et al. Single low dose primaquine to reduce gametocyte carriage and Plasmodium falciparum transmission after artemether-lumefantrine in children with asymptomatic infection: a randomised, double-blind, placebo-controlled trial. *BMC Med* 2016; **14**: 40.
50. Stone W, Mahamar A, Sanogo K, et al. Pyronaridine-artesunate or dihydroartemisinin-piperaquine combined with single low-dose primaquine to prevent Plasmodium falciparum malaria transmission in Ouelessebougou, Mali: a four-arm, single-blind, phase 2/3, randomised trial. *Lancet Microbe* 2022; **3**(1): e41-e51.
51. World Health Organization. Global Malaria Programme: WHO policy brief on single-dose primaquine as gametocytocide in *Plasmodium falciparum* malaria, 2015.
52. Baird JK, Hoffman SL. Primaquine therapy for malaria. *Clin Infect Dis* 2004; **39**(9): 1336-45.
53. Kane M. Primaquine Therapy and G6PD and CYP2D6 Genotype. In: Pratt VM, Scott SA, Pirmohamed M, Esquivel B, Kattman BL, Malheiro AJ, eds. Medical Genetics Summaries. Bethesda (MD); 2012.
54. Frampton JE. Tafenoquine: First Global Approval. *Drugs* 2018; **78**(14): 1517-23.
55. GlaxoSmithKline. Krintafel (tafenoquine) tablets, for oral use: US prescribing information. 2018.

56. Nuwa A, Baker K, Kajubi R, et al. Effectiveness of sulfadoxine-pyrimethamine plus amodiaquine and dihydroartemisinin-piperaquine for seasonal malaria chemoprevention in Uganda: a three-arm, open-label, non-inferiority and superiority, cluster-randomised, controlled trial. *Lancet Infect Dis* 2025.
57. Organization WH. Seasonal malaria chemoprevention with sulfadoxine–pyrimethamine plus amodiaquine in children: a field guide, 2023.
58. Dicko A, Roh ME, Diawara H, et al. Efficacy and safety of primaquine and methylene blue for prevention of Plasmodium falciparum transmission in Mali: a phase 2, single-blind, randomised controlled trial. *Lancet Infect Dis* 2018; **18**(6): 627-39.
59. Rueangweerayut R, Bancone G, Harrell EJ, et al. Hemolytic Potential of Tafenoquine in Female Volunteers Heterozygous for Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency (G6PD Mahidol Variant) versus G6PD-Normal Volunteers. *Am J Trop Med Hyg* 2017; **97**(3): 702-11.
60. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet* 2008; **371**(9606): 64-74.
61. Chen I, Diawara H, Mahamar A, et al. Safety of Single-Dose Primaquine in G6PD-Deficient and G6PD-Normal Males in Mali Without Malaria: An Open-Label, Phase 1, Dose-Adjustment Trial. *J Infect Dis* 2018; **217**(8): 1298-308.
62. Bastiaens GJH, Tiono AB, Okebe J, et al. Safety of single low-dose primaquine in glucose-6-phosphate dehydrogenase deficient falciparum-infected African males: Two open-label, randomized, safety trials. *PLoS One* 2018; **13**(1): e0190272.
63. Witmer K, Dahalan FA, Delves MJ, et al. Transmission of Artemisinin-Resistant Malaria Parasites to Mosquitoes under Antimalarial Drug Pressure. *Antimicrob Agents Chemother* 2020; **65**(1).
64. Barnes KI, Little F, Mabuza A, et al. Increased gametocytemia after treatment: an early parasitological indicator of emerging sulfadoxine-pyrimethamine resistance in falciparum malaria. *J Infect Dis* 2008; **197**(11): 1605-13.
65. Bousema T, Dinglasan RR, Morlais I, et al. Mosquito feeding assays to determine the infectiousness of naturally infected Plasmodium falciparum gametocyte carriers. *PLoS One* 2012; **7**(8): e42821.
66. Vanheer LNR, J.; Mahamar, A.; Smit, M.J.; Lanke, K.; Roh, M.E.; Sanogo, K.; Sinaba, Y.; Niambele, S.M.; Diallo, M.; Maguiraga, S.O.; Keita, S.; Samake, S.; Youssouf, A.; Diawara, H.; Traore, S.F.; Gosling, R.; Brown, J.M.; Drakeley, C.; Dicko, A.; Stone, W.; Bousema, T. The transmission blocking activity of artemisinin-combination, non-artemisinin, and 8-aminoquinoline antimalarial therapies: a pooled analysis of individual participant data. *medRxiv* 2024.
67. Dicko A, Brown JM, Diawara H, et al. Primaquine to reduce transmission of Plasmodium falciparum malaria in Mali: a single-blind, dose-ranging, adaptive randomised phase 2 trial. *Lancet Infect Dis* 2016; **16**(6): 674-84.
68. Mahamar A, Smit MJ, Sanogo K, et al. Artemether-lumefantrine with or without single-dose primaquine and sulfadoxine-pyrimethamine plus amodiaquine with or without single-dose tafenoquine to reduce Plasmodium falciparum transmission: a phase 2, single-blind, randomised clinical trial in Ouelessebougou, Mali. *Lancet Microbe* 2024; **5**(7): 633-44.
69. Mahamar AV, L.N.; Smit, M.J.; Sanogo, K.; Sinaba, Y.; Niambele, S.M.; et al. . Artemether-lumefantrine-amodiaquine or artesunate-amodiaquine combined with single low-dose primaquine to reduce Plasmodium falciparum malaria transmission in Ouélessébougou, Mali: a five-arm, phase 2, single-blind, randomised clinical trial. MedRXiv; 2024.

70. Stone W, Mahamar A, Smit MJ, et al. Single low-dose tafenoquine combined with dihydroartemisinin-piperaquine to reduce Plasmodium falciparum transmission in Ouelessebougou, Mali: a phase 2, single-blind, randomised clinical trial. *Lancet Microbe* 2022; **3**(5): e336-e47.

71. Cohee LM, Opondo C, Clarke SE, et al. Preventive malaria treatment among school-aged children in sub-Saharan Africa: a systematic review and meta-analyses. *Lancet Glob Health* 2020; **8**(12): e1499-e511.

72. Rek J, Blanken SL, Okoth J, et al. Asymptomatic School-Aged Children Are Important Drivers of Malaria Transmission in a High Endemicity Setting in Uganda. *J Infect Dis* 2022; **226**(4): 708-13.

73. Andolina C, Rek JC, Briggs J, et al. Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. *Lancet Infect Dis* 2021; **21**(11): 1568-78.

74. Stone W, Goncalves BP, Bousema T, Drakeley C. Assessing the infectious reservoir of falciparum malaria: past and future. *Trends Parasitol* 2015; **31**(7): 287-96.

75. Staedke SG, Maiteki-Sebuguzi C. Targeting malaria control to schoolchildren. *Lancet Glob Health* 2023; **11**(8): e1156-e7.

76. Walldorf JA, Cohee LM, Coalson JE, et al. School-Age Children Are a Reservoir of Malaria Infection in Malawi. *PLoS One* 2015; **10**(7): e0134061.

77. Rosenthal PJ. The interplay between drug resistance and fitness in malaria parasites. *Mol Microbiol* 2013; **89**(6): 1025-38.

78. World Health Organization. Tackling antimalarial drug resistance. Geneva, Switzerland, 2019.

79. Conrad MD, Asua V, Garg S, et al. Evolution of Partial Resistance to Artemisinins in Malaria Parasites in Uganda. *N Engl J Med* 2023; **389**(8): 722-32.

80. Gething PW, Smith DL, Patil AP, Tatem AJ, Snow RW, Hay SI. Climate change and the global malaria recession. *Nature* 2010; **465**(7296): 342-5.

81. World Health Organization. Emergence and spread of artemisinin resistance calls for intensified efforts to withdraw oral artemisinin monotherapy from the market. . Geneva, Switzerland., 2014.

82. Mairet-Khedim M, Leang R, Marmai C, et al. Clinical and In Vitro Resistance of Plasmodium falciparum to Artesunate-Amodiaquine in Cambodia. *Clin Infect Dis* 2021; **73**(3): 406-13.

83. Amaratunga C, Lim P, Suon S, et al. Dihydroartemisinin-piperaquine resistance in Plasmodium falciparum malaria in Cambodia: a multisite prospective cohort study. *Lancet Infect Dis* 2016; **16**(3): 357-65.

84. Phyo AP, Ashley EA, Anderson TJC, et al. Declining Efficacy of Artemisinin Combination Therapy Against P. Falciparum Malaria on the Thai-Myanmar Border (2003-2013): The Role of Parasite Genetic Factors. *Clin Infect Dis* 2016; **63**(6): 784-91.

85. World Health Organization. Multiple first-line therapies as part of the response to antimalarial drug resistance: an implementation guide. Geneva, 2024.

86. Nguyen TD, Olliaro P, Dondorp AM, et al. Optimum population-level use of artemisinin combination therapies: a modelling study. *Lancet Glob Health* 2015; **3**(12): e758-66.

87. Kabore JMT, Siribie M, Hien D, et al. Feasibility and Acceptability of a Strategy Deploying Multiple First-Line Artemisinin-Based Combination Therapies for Uncomplicated Malaria in the Health District of Kaya, Burkina Faso. *Trop Med Infect Dis* 2023; **8**(4).

88. Maiga H, Grivoyannis A, Sagara I, et al. Selection of pfcrt K76 and pfmdr1 N86 Coding Alleles after Uncomplicated Malaria Treatment by Artemether-Lumefantrine in Mali. *Int J Mol Sci* 2021; **22**(11).

89. Peto TJ, Tripura R, Callery JJ, et al. Triple therapy with artemether-lumefantrine plus amodiaquine versus artemether-lumefantrine alone for artemisinin-resistant, uncomplicated falciparum malaria: an open-label, randomised, multicentre trial. *Lancet Infect Dis* 2022; **22**(6): 867-78.

90. van der Pluijm RW, Tripura R, Hoglund RM, et al. Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated Plasmodium falciparum malaria: a multicentre, open-label, randomised clinical trial. *Lancet* 2020; **395**(10233): 1345-60.

91. Bassat Q, Maiga-Ascofare O, May J, et al. Challenges in the clinical development pathway for triple and multiple drug combinations in the treatment of uncomplicated falciparum malaria. *Malar J* 2022; **21**(1): 61.

92. Nguyen TD, Gao B, Amaratunga C, et al. Preventing antimalarial drug resistance with triple artemisinin-based combination therapies. *Nat Commun* 2023; **14**(1): 4568.

93. Group WGS. Gametocyte carriage in uncomplicated Plasmodium falciparum malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data. *BMC Med* 2016; **14**: 79.

94. Rakotoarisoa MA, Fenomanana J, Dodoson BT, Andrianaranjaka VHI, Ratsimbasoa A. Comparative effect of artemether-lumefantrine and artesunate-amodiaquine on gametocyte clearance in children with uncomplicated Plasmodium falciparum malaria in Madagascar. *Malar J* 2022; **21**(1): 331.

95. Adjuik M, Agnamey P, Babiker A, et al. Amodiaquine-artesunate versus amodiaquine for uncomplicated Plasmodium falciparum malaria in African children: a randomised, multicentre trial. *Lancet* 2002; **359**(9315): 1365-72.

96. de Jong RM, Tebeje SK, Meerstein-Kessel L, et al. Immunity against sexual stage Plasmodium falciparum and Plasmodium vivax parasites. *Immunol Rev* 2020; **293**(1): 190-215.

97. Sauerwein RW, Bousema T. Transmission blocking malaria vaccines: Assays and candidates in clinical development. *Vaccine* 2015; **33**(52): 7476-82.

98. Ponnudurai T, Lensen AH, Van Gemert GJ, Bensink MP, Bolmer M, Meuwissen JH. Infectivity of cultured Plasmodium falciparum gametocytes to mosquitoes. *Parasitology* 1989; **98 Pt 2**: 165-73.

99. Churcher TS, Blagborough AM, Delves M, et al. Measuring the blockade of malaria transmission--an analysis of the Standard Membrane Feeding Assay. *Int J Parasitol* 2012; **42**(11): 1037-44.

100. de Graaf H, Payne RO, Taylor I, et al. Safety and Immunogenicity of ChAd63/MVA Pfs25-IMX313 in a Phase I First-in-Human Trial. *Front Immunol* 2021; **12**: 694759.

101. Chichester JA, Green BJ, Jones RM, et al. Safety and immunogenicity of a plant-produced Pfs25 virus-like particle as a transmission blocking vaccine against malaria: A Phase 1 dose-escalation study in healthy adults. *Vaccine* 2018; **36**(39): 5865-71.

102. Talaat KR, Ellis RD, Hurd J, et al. Safety and Immunogenicity of Pfs25-EPA/Alhydrogel(R), a Transmission Blocking Vaccine against Plasmodium falciparum: An Open Label Study in Malaria Naive Adults. *PLoS One* 2016; **11**(10): e0163144.

103. Sagara I, Healy SA, Assadou MH, et al. Safety and immunogenicity of Pfs25H-EPA/Alhydrogel, a transmission-blocking vaccine against Plasmodium falciparum: a randomised, double-blind, comparator-controlled, dose-escalation study in healthy Malian adults. *Lancet Infect Dis* 2018; **18**(9): 969-82.

104. Carter R, Graves PM, Quakyi IA, Good MF. Restricted or absent immune responses in human populations to Plasmodium falciparum gamete antigens that are targets of malaria transmission-blocking antibodies. *J Exp Med* 1989; **169**(1): 135-47.

105. Healy SA, Anderson C, Swihart BJ, et al. Pfs230 yields higher malaria transmission-blocking vaccine activity than Pfs25 in humans but not mice. *J Clin Invest* 2021; **131**(7).

106. Sagara I, Healy SA, Assadou MH, et al. Malaria transmission-blocking vaccines Pfs230D1-EPA and Pfs25-EPA in Alhydrogel in healthy Malian adults; a phase 1, randomised, controlled trial. *Lancet Infect Dis* 2023; **23**(11): 1266-79.

107. Ko KT, Lennartz F, Mekhaiel D, et al. Structure of the malaria vaccine candidate Pfs48/45 and its recognition by transmission blocking antibodies. *Nat Commun* 2022; **13**(1): 5603.

108. Singh SK, Thrane S, Chourasia BK, et al. Pfs230 and Pfs48/45 Fusion Proteins Elicit Strong Transmission-Blocking Antibody Responses Against Plasmodium falciparum. *Front Immunol* 2019; **10**: 1256.

109. Singh SK, Roeffen W, Andersen G, et al. A Plasmodium falciparum 48/45 single epitope R0.6C subunit protein elicits high levels of transmission blocking antibodies. *Vaccine* 2015; **33**(16): 1981-6.

110. Singh SK, Roeffen W, Mistarz UH, et al. Construct design, production, and characterization of Plasmodium falciparum 48/45 R0.6C subunit protein produced in Lactococcus lactis as candidate vaccine. *Microb Cell Fact* 2017; **16**(1): 97.

111. Kundu P, Semesi A, Jore MM, et al. Structural delineation of potent transmission-blocking epitope I on malaria antigen Pfs48/45. *Nat Commun* 2018; **9**(1): 4458.

112. Theisen M, Soe S, Oeuvray C, et al. The glutamate-rich protein (GLURP) of Plasmodium falciparum is a target for antibody-dependent monocyte-mediated inhibition of parasite growth in vitro. *Infect Immun* 1998; **66**(1): 11-7.

113. Singh SK, Plieskatt J, Chourasia BK, et al. Preclinical development of a Pfs230-Pfs48/45 chimeric malaria transmission-blocking vaccine. *NPJ Vaccines* 2021; **6**(1): 120.

114. Tiono AB, Plieskatt JL, Ouedraogo A, et al. A randomized first-in-human phase I trial of differentially adjuvanted Pfs48/45 malaria vaccines in Burkinabe adults. *J Clin Invest* 2024; **134**(7).

115. Singh SK, Thrane S, Janitzek CM, et al. Improving the malaria transmission-blocking activity of a Plasmodium falciparum 48/45 based vaccine antigen by SpyTag/SpyCatcher mediated virus-like display. *Vaccine* 2017; **35**(30): 3726-32.

116. Scaria PV, Roth N, Schwendt K, et al. mRNA vaccines expressing malaria transmission-blocking antigens Pfs25 and Pfs230D1 induce a functional immune response. *NPJ Vaccines* 2024; **9**(1): 9.

117. Fougeroux C, Hagen SH, Goksoyr L, et al. A modular mRNA vaccine platform encoding antigen-presenting capsid virus-like particles enhances the immunogenicity of the malaria antigen Pfs25. *Nat Nanotechnol* 2025.

118. Kisalu NK, Idris AH, Weidle C, et al. A human monoclonal antibody prevents malaria infection by targeting a new site of vulnerability on the parasite. *Nat Med* 2018; **24**(4): 408-16.

119. Wang LT, Pereira LS, Flores-Garcia Y, et al. A Potent Anti-Malarial Human Monoclonal Antibody Targets Circumsporozoite Protein Minor Repeats and Neutralizes Sporozoites in the Liver. *Immunity* 2020; **53**(4): 733-44 e8.

120. Kayentao K, Ongoiba A, Preston AC, et al. Subcutaneous Administration of a Monoclonal Antibody to Prevent Malaria. *N Engl J Med* 2024; **390**(17): 1549-59.

121. Lyke KE, Berry AA, Mason K, et al. Low-dose intravenous and subcutaneous CIS43LS monoclonal antibody for protection against malaria (VRC 612 Part C): a phase 1, adaptive trial. *Lancet Infect Dis* 2023; **23**(5): 578-88.

122. Inklaar MR, de Jong RM, Bekkering ET, et al. Pfs230 Domain 7 is targeted by a potent malaria transmission-blocking monoclonal antibody. *NPJ Vaccines* 2023; **8**(1): 186.

123. Yu XQ, Robbie GJ, Wu Y, et al. Safety, Tolerability, and Pharmacokinetics of MEDI4893, an Investigational, Extended-Half-Life, Anti-Staphylococcus aureus Alpha-Toxin Human Monoclonal Antibody, in Healthy Adults. *Antimicrob Agents Chemother* 2017; **61**(1).

124. Gaudinski MR, Coates EE, Houser KV, et al. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. *PLoS Med* 2018; **15**(1): e1002493.

125. Robbie GJ, Criste R, Dall'acqua WF, et al. A novel investigational Fc-modified humanized monoclonal antibody, motavizumab-YTE, has an extended half-life in healthy adults. *Antimicrob Agents Chemother* 2013; **57**(12): 6147-53.

126. Kisalu NK, Pereira LD, Ernste K, et al. Enhancing durability of CIS43 monoclonal antibody by Fc mutation or AAV delivery for malaria prevention. *JCI Insight* 2021; **6**(3).

127. Gaudinski MR, Berkowitz NM, Idris AH, et al. A Monoclonal Antibody for Malaria Prevention. *N Engl J Med* 2021; **385**(9): 803-14.

128. Locke KW, Maneval DC, LaBarre MJ. ENHANZE((R)) drug delivery technology: a novel approach to subcutaneous administration using recombinant human hyaluronidase PH20. *Drug Deliv* 2019; **26**(1): 98-106.

129. Brickley EB, Coulibaly M, Gabriel EE, et al. Utilizing direct skin feeding assays for development of vaccines that interrupt malaria transmission: A systematic review of methods and case study. *Vaccine* 2016; **34**(48): 5863-70.

130. Coulibaly MB, Gabriel EE, Sinaba Y, et al. Optimizing Direct Membrane and Direct Skin Feeding Assays for Plasmodium falciparum Transmission-Blocking Vaccine Trials in Bancoumana, Mali. *Am J Trop Med Hyg* 2017; **97**(3): 719-25.

131. Eldering M, Bompard A, Miura K, et al. Comparative assessment of An. gambiae and An. stephensi mosquitoes to determine transmission-reducing activity of antibodies against P. falciparum sexual stage antigens. *Parasit Vectors* 2017; **10**(1): 489.

132. Ba EKC, Roh ME, Diallo A, et al. Effect of mass drug administration on malaria incidence in southeast Senegal during 2020-22: a two-arm, open-label, cluster-randomised controlled trial. *Lancet Infect Dis* 2025.

133. Buchwald AG, Sorkin JD, Sixpence A, et al. Association Between Age and Plasmodium falciparum Infection Dynamics. *Am J Epidemiol* 2019; **188**(1): 169-76.

134. Felger I, Maire M, Bretscher MT, et al. The dynamics of natural Plasmodium falciparum infections. *PLoS One* 2012; **7**(9): e45542.

135. Blanken SL, Kilama M, Ramjith J, et al. Anopheles mosquito exposure is associated with age, gender and bed net use in areas in Uganda experiencing varying malaria transmission intensity. *medRxiv* 2024.

136. Challenger JD, van Beek SW, Ter Heine R, et al. Modeling the Impact of a Highly Potent Plasmodium falciparum Transmission-Blocking Monoclonal Antibody in Areas of Seasonal Malaria Transmission. *J Infect Dis* 2023; **228**(2): 212-23.

137. Jefferson M Jones KEF-D, Mila M Prill, Lauren E Roper, Oliver Brooks, Pablo J Sánchez, Camille N Kotton, Barbara E Mahon, Sarah Meyer, Sarah S Long, Meredith L McMorrow. Use of Nirsevimab for the Prevention of Respiratory Syncytial Virus Disease Among Infants and Young Children: Recommendations of the Advisory Committee on Immunization Practices – United States, 2023. *MMWR Morb Mortal Wkly Rep* 2023.

138. Garegnani L, Styrmisdottir L, Roson Rodriguez P, Escobar Liquitay CM, Esteban I, Franco JV. Palivizumab for preventing severe respiratory syncytial virus (RSV) infection in children. *Cochrane Database Syst Rev* 2021; **11**(11): CD013757.

139. Rogers DJ, Randolph SE. Climate change and vector-borne diseases. *Adv Parasitol* 2006; **62**: 345-81.

140. Colon-Gonzalez FJ, Sewe MO, Tompkins AM, et al. Projecting the risk of mosquito-borne diseases in a warmer and more populated world: a multi-model, multi-scenario intercomparison modelling study. *Lancet Planet Health* 2021; **5**(7): e404-e14.

141. Adshead F, Al-Shahi Salman R, Aumonier S, et al. A strategy to reduce the carbon footprint of clinical trials. *Lancet* 2021; **398**(10297): 281-2.

142. Tao Y, Steckel D, Klemes JJ, You F. Trend towards virtual and hybrid conferences may be an effective climate change mitigation strategy. *Nat Commun* 2021; **12**(1): 7324.

143. Barksby R. 1st Women in Malaria conference. *Lancet Infect Dis* 2021; **21**(5): 607.

144. Bousema T, Selvaraj P, Djimde AA, et al. Reducing the Carbon Footprint of Academic Conferences: The Example of the American Society of Tropical Medicine and Hygiene. *Am J Trop Med Hyg* 2020; **103**(5): 1758-61.

145. London School of Hygiene & Tropical Medicine. MRCG at LSHTM Inaugurates Biggest Solar Power Generation System in The Gambia. 2019.

146. Fox L. MRC-NIHR TMRP Greener Trials Working Group Remit. 2025. https://methodologyhubs.mrc.ac.uk/about/working-groups/trial-conductwg/tcwg-subgroup-greener-trials/ (accessed 24-05 2025).

147. Griffiths J, Fox L, Williamson PR, Low Carbon Clinical Trials G. Quantifying the carbon footprint of clinical trials: guidance development and case studies. *BMJ Open* 2024; **14**(1): e075755.

APPENDIX

Summary

Malaria remains one of the most persistent global health challenges, with *Plasmodium falciparum* causing the highest morbidity and mortality rates, particularly in sub-Saharan Africa. With an estimated 263 million malaria cases and 597.000 malaria-related deaths in 2023–73.7% of which were in children under five–the global malaria burden remains high. Despite concerted efforts over the past two decades, progress in reducing the malaria burden has stalled due to limited coverage of existing tools, stagnating funding, and rising resistance to insecticides, drugs, and diagnostics. This stagnation underlines the need for novel interventions that not only treat malaria but also interrupt its transmission cycle.

In the first part of this thesis, we evaluated how different antimalarial regimens impact *P. falciparum* transmission potential by conducting three phase 2 randomised clinical trials in Ouélessébougou, Mali. In **Chapters 2, 3A, and 4**, we explored how different artemisinin-based combination therapies (ACTs) and non-ACT regimens affect gametocyte carriage and how this translates into transmission reduction. We also assessed whether adding single low doses of gametocytocidal drugs to standard antimalarial regimens enhances the clearing of *P. falciparum* gametocytes and reduces *P. falciparum* transmission. Specifically, we tested combinations of dihydroartemisinin–piperaquine with tafenoquine (**Chapter 2**), artemether–lumefantrine or sulfadoxine–pyrimethamine plus amodiaquine with or without primaquine or tafenoquine (**Chapter 3A**), and triple artemisinin-based combination therapies with or without primaquine (**Chapter 4**).

A key methodological strength in our studies was the use of direct membrane feeding assays (DMFA) both before and after treatment to assess infectiousness to mosquitoes. This approach is important because relying solely on gametocyte quantification can be misleading; gametocyte density does not always correlate with the actual potential to infect mosquitoes. By performing DMFA at multiple time points, we were able to directly measure the impact of treatment on transmission potential, providing a more reliable and comprehensive assessment of transmission reduction. While ACTs remain central to malaria treatment by reducing parasite load and clinical symptoms, we demonstrated that their transmission-reducing efficacy varies. Our findings showed that artemether–lumefantrine has strong transmission-blocking activity, whereas dihydroartemisinin–piperaquine and amodiaquine–artesunate have a more

modest impact. Future strategies should optimise both cure and transmission reduction by adding gametocytocidal drugs such as low-dose primaquine or tafenoquine, especially in low-transmission or artemisinin-resistant settings. Refining seasonal malaria chemoprevention to include gametocytocidal agents and targeting school-aged children could further reduce the infectious reservoir. Combining effective treatments with targeted transmission-blocking measures and broader coverage could support sustained malaria control.

The second part of this thesis focuses on active and passive immunisation aimed at blocking *P. falciparum* transmission. In **Chapter 5**, we evaluated the transmission-blocking vaccine candidate R0.6C for the first time in humans. R0.6C is a fusion protein consisting of Pfs48/45 domain 3 (6C) and the N-terminal region of *P. falciparum* glutamate-rich protein (R0). We demonstrated in a phase 1 trial that R0.6C is safe and immunogenic in malaria-naïve adults, inducing functional transmission-blocking antibodies. However, antibody titres and serum concentrations were insufficient to achieve potent transmission-reducing activity in standard membrane feeding assays (SMFA) using neat serum samples. Purified vaccine-specific immunoglobulins, on the other hand, achieved up to 99% transmission-reducing activity, highlighting the potential of this vaccine construct while underlining the need for further optimisation of immunogenicity and dosing strategies.

In **Chapter 6**, we performed the first clinical assessment of the humanised *P. falciparum* transmission-reducing monoclonal antibody TB31F, which binds the gamete surface protein Pfs48/45. Administered as a single intravenous or subcutaneous dose, TB31F was well tolerated across all tested dosages. Importantly, serum concentrations achieved following a 10 mg/kg intravenous dose maintained over 80% transmission-reducing activity in SMFA for an estimated 160 days. These findings demonstrate the high potency and prolonged efficacy of TB31F, positioning monoclonal antibodies as a promising tool in malaria elimination programmes, particularly in areas with seasonal transmission. Building on these findings, we reflect on the broader role of monoclonal antibodies in malaria prevention in **Chapter 7**. Monoclonal antibodies offer the advantage of predictable pharmacokinetics and defined specificity and could be integrated into existing malaria control strategies, for example through targeted seasonal deployment or as a bridge to more broadly applicable vaccination strategies.

Recognising that clinical research itself has environmental impacts, we incorporated a life cycle assessment (LCA) of a malaria clinical trial as part of this thesis (**Chapter 3B**). This LCA, focusing on the trial described in **Chapter 3A**, quantified greenhouse gas emissions and other environmental burdens associated with trial conduct, including transportation, electricity use, laboratory consumables, and intercontinental shipments. The analysis revealed that international staff travel and electricity consumption in Mali were the primary contributors to the trial's carbon footprint, accounting for approximately 50% and 28% of total emissions, respectively. Laboratory consumables, although impactful in terms of land and water use, contributed minimally to greenhouse gas emissions. We identified several actionable strategies to reduce the environmental impact of clinical trials, such as using energy-efficient laboratory equipment, implementing alternative sample transport methods, and reducing intercontinental travel through attending hybrid conferences.

In the final part of this thesis, we contributed to the first clinical evaluation of a modular capsid virus-like particle (cVLP) platform through a phase 1 trial of the SARS-CoV-2 vaccine candidate ABNCoV2 (**Chapter 8**). This study provided proof-of-concept for the safety, tolerability, and immunogenicity of the cVLP platform. Vaccination with ABNCoV2 elicited robust antibody responses and functional virus-neutralising activity against several SARS-CoV-2 variants, although responses were somewhat lower against the Omicron BA.1 variant. The lessons learnt in **Chapter 8** underline that the cVLP platform holds promise beyond COVID-19. A cVLP-based version of the placental malaria vaccine PAMVAC is currently under development, with clinical testing scheduled for 2025. Beyond malaria, cVLP vaccines are being explored for other infectious diseases, including Nipah, Marburg, and Sudan viruses. These developments illustrate how cVLP technology is expanding its relevance beyond a single disease.

Collectively, the studies presented in this thesis contribute to the global effort to reduce *P. falciparum* transmission through a comprehensive evaluation of both established and emerging strategies. By consistently applying mosquito feeding assays and harmonised methodologies, we provided robust insights into the transmission-reducing efficacy of antimalarials, gametocytocidal agents, a transmission-blocking vaccine, and a monoclonal antibody. We also highlight the importance of integrating transmission-blocking tools into existing malaria control programmes, tailored to local transmission dynamics

and resistance patterns. In addition, we addressed broader challenges by incorporating sustainability considerations into research practices and expanding the technological toolkit for vaccine development.

Samenvatting

Malaria blijft wereldwijd een van de meest voortdurende gezondheids-problemen, waarbij *Plasmodium falciparum* verantwoordelijk is voor de hoogste morbiditeit en mortaliteit, met name in sub-Sahara Afrika. Met naar schatting 263 miljoen malariagevallen en 597.000 malaria-gerelateerde sterfgevallen in 2023 – waarvan 73,7% bij kinderen onder de vijf jaar – blijft de wereldwijde ziektelast van malaria hoog. Ondanks gezamenlijke inspanningen in de afgelopen twee decennia is de vooruitgang in het terugdringen van malaria gestagneerd. Dit komt onder andere als gevolg van beperkte dekking van bestaande interventies, stagnerende financiering en toenemende resistentie tegen insecticiden, geneesmiddelen en diagnostische hulpmiddelen. Deze stagnatie onderstreept de noodzaak van nieuwe interventies die niet alleen malaria behandelen, maar ook de transmissie van de malariaparasiet onderbreken.

In het eerste deel van dit proefschrift onderzochten we hoe verschillende antimalariamiddelen de transmissiepotentie van *P. falciparum* beïnvloeden door drie fase 2 gerandomiseerde klinische studies uit te voeren in Ouélessébougou, Mali. In **hoofdstukken 2, 3A en 4** onderzochten we hoe verschillende artemisinine-combinatietherapieën (ACT's) en niet-ACT-regimes de gametocytenlast beïnvloeden en hoe dit zich vertaalt naar transmissiereductie. We evalueerden ook of toevoeging van een enkele lage dosis gametocytocidale middelen aan standaard antimalariabehandelingen de klaring van *P. falciparum*-gametocyten verbetert en de transmissie van *P. falciparum* vermindert. Concreet testten we combinaties van dihydroartemisinine/piperaquine met verschillende doseringen tafenoquine (**hoofdstuk 2**), artemether/lumefantrine met of zonder primaquine en sulfadoxine–pyrimethamine plus amodiaquine met of zonder tafenoquine (**hoofdstuk 3A**), en triple artemisinine-combinatietherapieën met of zonder primaquine (**hoofdstuk 4**).

Een belangrijke methodologische sterkte van onze studies was het gebruik van direct membrane feeding assays (DMFA) vóór en na behandeling om transmissiepotentieel te meten. Dit is essentieel, aangezien het vertrouwen op de aanwezigheid van gametocyten alleen misleidend kan zijn; de gametocytendichtheid komt niet altijd overeen met het daadwerkelijke infectiepotentieel voor muggen. Door DMFA op meerdere tijdstippen uit te voeren, konden we het effect van behandeling op transmissie rechtstreeks

vaststellen, wat een betrouwbaardere en meer uitgebreide beoordeling van transmissiereductie mogelijk maakte.

Hoewel artemisinecombinatietherapieën (ACT's) de kern blijven vormen van de malariabehandeling vanwege hun vermogen om parasietenlast en klinische symptomen te verminderen, laten onze resultaten zien dat het transmissiereducerende effect van verschillende ACT's aanzienlijk verschilt. Zo vertoonde artemether–lumefantrine een sterk transmissieblokkerend effect, terwijl dihydroartemisinine–piperaquine en amodiaquine–artesunaat een meer bescheiden werking lieten zien. Toekomstige strategieën zouden zich dan ook moeten richten op het optimaliseren van zowel klinische genezing als transmissiereductie. Dit kan worden bereikt door het toevoegen van gametocytocidale middelen zoals lage doses primaquine of tafenoquine, met name in gebieden met lage transmissie of opkomende artemisinineresistentie. Het verfijnen van seizoensgebonden malariapreventie (SMC) – bijvoorbeeld door gametocytociden toe te voegen en de focus te leggen op schoolgaande kinderen – zou het infectieuze reservoir verder kunnen verkleinen. Een gecombineerde aanpak met effectieve behandelingen, gerichte transmissieblokkerende maatregelen en bredere implementatie kan zo bijdragen aan een duurzamere malariacontrole.

Het tweede deel van dit proefschrift richt zich op zowel actieve als passieve immunisatiestrategieën die erop gericht zijn de transmissie van *P. falciparum* te blokkeren. In **hoofdstuk 5** onderzochten we voor het eerst bij mensen het transmissieblokkerende vaccin R0.6C, een fusie-eiwit bestaande uit domein 3 van Pfs48/45 (6C) en het N-terminale deel van het glutamaatrijke eiwit van *P. falciparum* (R0). In deze fase 1-studie bij malaria-naïeve volwassenen bleek R0.6C veilig en immunogeen, met de inductie van functionele, transmissieblokkerende antilichamen. Desondanks waren de opgewekte antilichaamtiters en serumconcentraties ontoereikend om in standaard membraanvoedingsassays (SMFA) met onbewerkt serum een sterke transmissiereductie te realiseren. Gezuiverde immunoglobulinen behaalden daarentegen tot 99% transmissiereductie, wat het potentieel van dit vaccin bevestigt, maar ook het belang benadrukt van verdere optimalisatie van de immunogeniciteit en doseringsstrategie.

In **hoofdstuk 6** beschrijven we de eerste klinische evaluatie van het gehumaniseerde, transmissiereducerende monoklonale antilichaam TB31F, dat gericht is tegen het gametenoppervlakte-eiwit Pfs48/45. Toegediend als

eenmalige intraveneuze of subcutane dosis werd TB31F goed verdragen bij alle onderzochte doseringen. Cruciaal is dat een intraveneuze dosis van 10 mg/kg gedurende ongeveer 160 dagen in staat was om meer dan 80% transmissie reducerende activiteit in SMFA te handhaven. Deze resultaten onderstrepen het sterke potentieel en de langdurige werking van TB31F, waarmee monoklonale antilichamen zich profileren als veelbelovende interventies binnen malariabestrijdingsprogramma's, in het bijzonder in regio's met seizoensgebonden transmissie.

Voortbouwend op deze bevindingen bespreekt **hoofdstuk 7** de bredere rol van monoklonale antilichamen in malariapreventie. Naast hun inzet als op zichzelf staande interventie wordt ook de combinatie van een beschermend en een transmissiereducerend antilichaam, zoals CIS43LS en TB31F, besproken. Gerichte, seizoensgebonden toediening of gebruik als overbrugging tot bredere vaccinatieprogramma's biedt hierbij interessante mogelijkheden.

Erkennend dat klinisch onderzoek zelf milieueffecten met zich meebrengt, omvat dit proefschrift ook een levenscyclusanalyse (LCA) van een malariastudie (**hoofdstuk 3B**). Deze analyse, uitgevoerd op de studie uit **hoofdstuk 3A**, bracht de uitstoot van broeikasgassen en andere milieubelasingen in kaart die samenhangen met onder meer transport, elektriciteitsverbruik, laboratoriumartikelen en intercontinentale verschepingen. De grootste bijdragen aan de carbon footprint kwamen van internationale reizen van personeel (ca. 50%) en elektriciteitsverbruik in Mali (ca. 28%). Hoewel laboratoriummaterialen relevant waren voor land- en watergebruik, droegen ze weinig bij aan de broeikasgasuitstoot. De analyse leverde praktische strategieën op om de milieu-impact van klinische studies te beperken, zoals energiezuinige apparatuur, alternatieve transportopties en het beperken van vliegreizen door deelname aan hybride conferenties.

In het laatste deel van dit proefschrift werkten we aan de eerste klinische evaluatie van een modular capsid-virus-like particles (cVLP)-platform, via een fase 1-studie van het SARS-CoV-2-vaccin ABNCoV2 (**hoofdstuk 8**). De resultaten toonden aan dat het vaccin veilig, goed verdraagbaar en immunogeen was, met sterke antilichaam- en virusneutraliserende responsen tegen diverse SARS-CoV-2-varianten, zij het iets lager tegen Omicron BA.1. Deze bevindingen onderstrepen de bredere potentie van het cVLP-platform. Een cVLP-gebaseerde versie van het placentaire malariavaccin PAMVAC is momenteel in ontwikkeling, met klinische testen gepland voor 2025. Naast

malaria wordt cVLP-technologie onderzocht voor andere infectieziekten, waaronder Nipah-, Marburg- en Sudan-virussen. Deze ontwikkelingen illustreren hoe de voor COVID-19 geteste cVLP-technologie breder inzetbaar is voor de preventie van andere infectieziekten.

De in dit proefschrift gepresenteerde studies dragen gezamenlijk bij aan de wereldwijde inspanningen om *P. falciparum*-transmissie te verminderen, via een brede evaluatie van zowel bestaande als innovatieve interventiestrategieën. Door het consistente gebruik van muggenvoedingsassays en geharmoniseerde methodologieën hebben we robuuste inzichten verkregen in de transmissiereducerende effectiviteit van antimalariamiddelen, gametocytocidale middelen, een transmissieblokkerend vaccin en een monoklonaal antilichaam. We onderstrepen het belang van het integreren van transmissieblokkerende interventies in malariabestrijdingsprogramma's, afgestemd op lokale transmissiepatronen en resistentie. Tot slot hebben we bredere uitdagingen aangepakt door duurzaamheidsoverwegingen te integreren in onderzoekspraktijken en het technologische instrumentarium voor vaccinontwikkeling uit te breiden.

Research Data Management

This thesis is based on the results of medical-scientific research involving human and non-human data, conducted during my PhD at the Department of Medical Microbiology, Radboud University Medical Center (Radboudumc). All studies were conducted in accordance with relevant national and international legislation, ICH-GCP guidelines, and Radboudumc policies. Research data were managed to enhance findability, accessibility, interoperability, and reusability (FAIR principles).

Ethics and Privacy

The studies described in Chapters 2, 3A, 4, 5, 6, and 8 were subject to the Dutch Medical Research Involving Human Subjects Act (WMO). Ethical approval was obtained as follows:

- Chapters 2, 3A, and 4: Ethics Committee of the Faculty of Medicine, Pharmacy, and Dentistry, University of Science, Techniques, and Technologies of Bamako, Mali, and the Research Ethics Committee of the London School of Hygiene & Tropical Medicine, UK (reference numbers: 21905, 26257, 28014).
- Chapters 5 and 8: Central Committee on Research Involving Human Subjects (CCMO), the Netherlands (file numbers: NL76664.000.21, NL76192.000.20).
- Chapter 6: METC Oost-Nederland (file number: NL69779.091.19).

Informed consent was obtained from all participants and/or their legal guardians, including consent for data collection and processing. For Chapters 5, 6, and 8, reuse of data for future research is only possible after participant consent.

To safeguard privacy, pseudonymization measures were applied. Pseudonymization keys were securely stored separately from research data, with access restricted to authorised project members. Technical and organisational safeguards were implemented to ensure availability, integrity, and confidentiality of all data, including independent monitoring, access authorisation, and secure storage.

Data Collection and Storage

Data were managed in compliance with ICH-GCP guidelines and institutional policies. Paper records were stored in locked, fireproof cabinets with access restricted to authorised individuals.

Processed and raw datasets are stored securely on the department server, accessible to authorised lab members and backed up daily on the Radboudumc server. Essential study documentation, including protocols and monitoring reports, was archived according to institutional and regulatory requirements.

Data Sharing and FAIR Principles

All studies described in this thesis are published open access. The data from Chapters 2, 3A, and 4 are available through the open-access clinical epidemiology database ClinEpiDB under the study names NECTAR2, NECTAR3, and NECTAR 4, respectively (https://clinepidb.org). The dataset supporting Chapter 3B is available in the online supplementary material accompanying the published article. The datasets from Chapters 5, 6, and 8 are published with restricted access in Radboud Data Repository. Requests for access will be checked by the data steward of the dataset, against the conditions for sharing the data as described in the signed Informed Consent.

PhD Portfolio

Department: **Medical Microbiology**
PhD period: **01/09/2020 – 30/04/2024**
PhD Supervisor(s): **Prof. J.T. Bousema, Prof. B.G. Mordmüller**
PhD Co-supervisor(s): **Dr M.B.B. McCall**

Training activities	Hours
Courses	
– Radboudumc - Introduction day (2020)	6.00
– Radboudumc - eBROK course (for Radboudumc researchers working with human subjects) (2020)	26.00
– RIHS - Introduction course for PhD candidates (2021)	15.00
– Clinical Epidemiology Course (2021)	42.00
– COVID-19 Vaccine Development & Implementation workshop (2021)	6.00
– Radboudumc - Scientific integrity (2022)	20.00
– Zeroverse carbon literacy (2022)	14.00
– How to sell your science (2022)	4.00
– Introduction in using R (2023)	14.00
– Radboudumc - e-learning Human-related scientific research in Radboudumc (2025)	2.00
– ICH Good Clinical Practice E6(R3) (2025)	6.00
Seminars	
– Models and methods for infectious diseases (2023)	2.00
– Malaria Gordon Research Seminar (poster presentation) (2023)	28.00
– Research Integrity Round (2023)	4.00
– Structural and Molecular Mechanisms of Plasmodium vivax invasion (2023)	2.00
– Dutch Malaria Day (oral presentation) (2023)	14.00
Conferences	
– American Society of Tropical Medicine and Hygiene – Annual Meeting (2020) – hybrid, attended online	42.00
– Women in Malaria conference (oral presentation) (2021)	42.00
– American Society of Tropical Medicine and Hygiene – Annual Meeting (2021) – hybrid, attended online	42.00
– Johns Hopkins - Future of Malaria Research Symposium (poster presentation) (2022) – hybrid, attended online	28.00
– American Society of Tropical Medicine and Hygiene – Annual Meeting (oral presentation) (2022) – hybrid, attended online	56.00
– Scientific Spring Meeting of the Dutch Society of Medical Microbiology (NVMM) (oral presentation) (2023)	28.00
– Malaria Gordon Research Conference (poster presentation) (2023)	56.00
– Multilateral Initiative on Malaria (MIM) Society 8th Pan-African Malaria Conference (oral presentation) (2024)	71.00
– Women in Malaria conference (2025)	16.00
Other	
– Research Integrity Round (2022)	2.00
– Radboud Science Day (2022)	14.00
– Supervision bachelor student (2023)	56.00
– Supervision bachelor student (2023)	56.00
– PhD retreat (oral presentation) (2023)	16.00
– PhD retreat (2024)	28.00

Teaching activities	
Lecturing	
– Minor Global Health Study (2022)	16.00
– Minor Global Health Study (2023)	16.00
– Minor Global Health Study (2024)	16.00
Supervision of internships / other	
– Supervision medical student (2021)	28.00
– Supervision bachelor students (2022)	42.00
Total	**876.00**

About the author

Merel Smit was born on 9 August 1995 in Liempde, the Netherlands, to Willemien Lenders and Frank Smit. At just one month old, she boarded her first flight to Ouagadougou, Burkina Faso. She spent her early childhood in Ouagadougou and Koudougou, together with her brother Thierry and sister Robin. The family later moved to Nouakchott, Mauritania, and returned to the Netherlands after four years. Merel attended primary school in Cadier & Keer and, at the age of eleven, moved once more to Niamey, Niger, where she spent a year at the French Lycée La Fontaine.

After graduating from Porta Mosana College in Maastricht in 2013, she moved to Nijmegen to study Medicine at Radboud University. During her studies, she became active in the International Federation of Medical Students' Associations (IFMSA) in Nijmegen, where she served as treasurer. After completing her bachelor's degree, she attended a tropical infectious diseases course in Sudan. During her master's, she completed clinical internships in Aruba and Indonesia. Her interest in infectious diseases and research deepened during a scientific internship on malaria at the Department of Medical Microbiology under the supervision of Dr Foekje Stelma. It was during this internship that she met Dr Matthew McCall, who would later become her daily supervisor and co-promotor during her PhD.

In 2020, she began her PhD in the malaria research group at Radboudumc, under the supervision of Dr Matthew McCall, Prof Teun Bousema, and Prof Benjamin Mordmüller. As part of her PhD, she conducted five clinical malaria trials–two in the Netherlands and three in Mali, the latter in collaboration with the Parasites & Microbes Research and Training Center (PMRTC) in Mali and the London School of Hygiene and Tropical Medicine (LSHTM). She also led a first-in-human SARS-CoV-2 vaccine trial in the Netherlands during the COVID-19 pandemic and conducted a life cycle assessment to examine the environmental impact of clinical research. Additionally, she spent eight months at the Centre de Recherches Médicales de Lambaréné (CERMEL) in Gabon, where she carried out a study on the relationship between malaria and sleep.

After completing her PhD, Merel moved to Gabon and continued her work as a clinical malaria researcher. She currently coordinates a phase 1/2a clinical trial of the monoclonal antibody TB31F in Mali, an intervention she had previously investigated in a phase 1 trial during her PhD. In parallel, she is working on a novel study assessing the potential of the endectocide lotilaner, continuing her focus on *Plasmodium falciparum* transmission and malaria elimination efforts.

List of publications

Publications in this thesis

1. **Merel J. Smit***, Almahamoudou Mahamar*, Emma Kooistra, Kjerstin Lanke, Koualy Sanogo, Patrick Wilikpan Okedy, Mohamed A. Yehia, Chris Drakeley, Hugo Touw, Will Stone, Alassane Dicko, Teun Bousema†, Tim Stobernack†. Life cycle assessment of a clinical malaria trial in Mali reveals large environmental impacts of electricity consumption and international travel. *PLOS Sustainability and Transformation.* 2025 February 28.
2. Almahamoudou Mahamar*, Leen N. Vanheer*, **Merel J. Smit**, Koualy Sanogo, Youssouf Sinaba, Sidi M. Niambele, Makonon Diallo, Oumar M. Dicko, Richard S. Diarra, Seydina O. Maguiraga, Ahamadou Youssouf, Adama Sacko, Sekouba Keita, Siaka Samake, Adama Dembele, Karina Teelen, Yahia Dicko, Sekou F. Traore, Arjen Dondorp, Chris Drakeley†, Will Stone†, Alassane Dicko†. Artemether-lumefantrine-amodiaquine or artesunate-amodiaquine combined with single low-dose primaquine to reduce *Plasmodium falciparum* malaria transmission in Ouélessébougou, Mali: a five-arm, phase 2, single-blind, randomised clinical trial. *The Lancet Microbe*. 2024 December 17.
3. Almahamoudou Mahamar*, **Merel J. Smit***, Koualy Sanogo, Youssouf Sinaba, Sidi M. Niambele, Adama Sacko, Oumar M. Dicko, Makonon Diallo, Seydina O. Maguiraga, Yaya Sankaré, Sekouba Keita, Siaka Samake, Adama Dembele, Kjerstin Lanke, Rob ter Heine, John Bradley, Yahia Dicko, Sekou F. Traore, Chris Drakeley†, Alassane Dicko†, Teun Bousema†, Will Stone†. Artemether-lumefantrine with or without single-dose primaquine and sulfadoxine-pyrimethamine plus amodiaquine with or without single-dose tafenoquine to reduce *Plasmodium falciparum* transmission: a phase 2 single-blind randomised clinical trial in Ouelessebougou, Mali. *The Lancet Microbe.* 2024 May 1.
4. Manon Alkema, **Merel J. Smit**, Catherin Marin-Mogollon*, Koen Totté*, Karina Teelen, Geert-Jan van Gemert, Marga van de Vegte-Bolmer, Benjamin G. Mordmüller, Jenny M. Reimer, Karin L. Lövgren Bengtsson, Robert W. Sauerwein, Teun Bousema, Jordan Plieskatt, Michael Theisen, Matthijs M. Jore, Matthew B.B. McCall. A Pfs48/45-based vaccine to block *Plasmodium falciparum* transmission: a phase 1, open-label, clinical trial. *BMC medicine.* 2024 April 23.

5. **Merel J. Smit,** Matthew B.B. McCall. Monoclonals against malaria: the promise of passive protection. [Comment] *The Lancet Infectious Diseases*. 2023 January 25.
6. **Merel J. Smit***, Adam F Sander*, Maud B.P.A. Ariaans, Cyrielle Fougeroux, Constanze Heinzel, Rolf Fendel, Meral Esen, Peter G. Kremsner, Rob ter Heine, Heiman F. Wertheim, Manja Idorn, Søren Riis Paludan, Alexander P. Underwood, Alekxander Binderup, Santseharay Ramirez, Jens Bukh, Max Soegaard, Sayit M. Erdogan, Tobias Gustavsson, Stine Clemmensen, Thor G Theander, Ali Salanti, Mette Hamborg, Willem A. de Jongh, Matthew B.B. McCall, Morten A. Nielsen†, Benjamin G. Mordmüller†, on behalf of the COUGH-1 trial study group. First-in-human use of a modular capsid virus-like vaccine platform: an open-label, non-randomised, phase 1 clinical trial of the SARS-CoV-2 vaccine ABNCoV2. *The Lancet Microbe*. 2023 January 18.
7. Saskia C. van der Boor*, **Merel J. Smit***, Stijn W. van Beek, Jordache Ramjith, Karina Teelen, Marga van de Vegte-Bolmer, Geert-Jan van Gemert, Peter Pickkers, Yimin Wu, Emily Locke, Shwu-Maan Lee, John Aponte, C. Richter King, Ashley J. Birkett, Kazutoyo Miura, Morolayo A. Ayorinde, Robert W. Sauerwein, Rob ter Heine, Christian F. Ockenhouse, Teun Bousema, Matthijs M. Jore†, Matthew B.B. McCall†. Safety, tolerability, and *Plasmodium falciparum* transmission-reducing activity of monoclonal antibody TB31F: a single-centre, open-label, first-in-human, dose-escalation, phase 1 trial in healthy malaria-naive adults. *The Lancet Infectious Diseases.* 2022 August 10.
8. Will Stone*, Almahamoudou Mahamar*, **Merel J. Smit***, Koualy Sanogo, Youssouf Sinaba, Sidi M. Niambele, Adama Sacko, Sekouba Keita, Oumar M. Dicko, Makonon Diallo, Seydina O. Maguiraga, Siaka Samake, Oumar Attaher, Kjerstin Lanke, Rob ter Heine, John Bradley, Matthew B.B. McCall, Djibrilla Issiaka, Sekou F. Traore, Teun Bousema†, Chris Drakeley†, Alassane Dicko†. Single low-dose tafenoquine combined with dihydroartemisinin–piperaquine to reduce *Plasmodium falciparum* transmission in Ouelessebougou, Mali: a phase 2, single-blind, randomised clinical trial. *The Lancet Microbe*. 2022 March 23.

Publications not in this thesis

9. Jeroen Bok*, Estefania Martínez-Albert*, Jean-Claude Dejón-Agobe*, **Merel J. Smit**, Christian Ascorbate, Bibi Muusz, Markus Gmeiner, Catherin Marin Mogollon, Karina Teelen, Andreas Peter, Tanja Lange, Stoyan Dimitrov, Ayôla Akim Adegnika, Benjamin Mordmüller, Luciana Besedovsky. Asymptomatic *Plasmodium falciparum* malaria infection enhances slow-wave sleep in humans. *Manuscript in preparation for submission to Science Translational Medicine*.
10. Sara Lynn Blanken, Maartje Inklaar, Zhong Wan, Felix Evers, **Merel J. Smit**, Vladyslav Kalyuzhnyy, Julie Verhoef, Ezra Bekkering, Michelle Schinkel, Saskia Mulder, Carolina Andrade, Geert-Jan van Gemert, Thomas H.A. Ederveen, Patrick Zeeuwen, Alem Gusinac, Teun Bousema, Felix J.H. Hol. Blood, sweat, and beers: investigating mosquito biting preferences amidst noise and intoxication in a cross-sectional cohort study at a large music festival. *Under review at BMJ*.
11. Wouter Graumans*, Will J. R. Stone*, Jordache Ramjith, Chiara Andolina, Carla Proietti, Sarah Merkling, Ronald van Rij, **Merel J. Smit**, Heiman Wertheim, Teun Bousema. Justifiably tight regulations? On wearing swimming briefs for hygiene reasons. *Submitted to Travel Medicine & Infectious Diseases.*
12. Leen N. Vanheer*, Jordache Ramjith*, Almahamoudou Mahamar*, **Merel J. Smit**, Koualy Sanogo, Kjerstin Lanke, Michelle E. Roh, Koualy Sanogo, Youssouf Sinaba, Sidi M. Niambele, Makonon Diallo, Seydina O. Maguiraga, Sekouba Keita, Ahamadou Youssouf, Halimatou Diawara, Sekou F. Traore, Roly Gosling, Joelle M. Brown, Chris Drakeley, Alassane Dicko†, Will Stone†, Teun Bousema†. The transmission blocking activity of artemisinin-combination, non-artemisinin, and 8-aminoquinoline antimalarial therapies: a pooled analysis of individual participant data. *PLOS Medicine.* 2025 August 14.
13. Julie Van Coillie, Tamas Pongracz, Tonci Sustic, Wenjun Wang, Jan Nouta, Mathieu Le Gars, Sofie Keijzer, Federica Linty, Olvi Cristianawati, Jim B.D. Keijser, Remco Visser, Lonneke A. van Vught, Marleen A. Slim, Niels van Mourik, **Merel J. Smit**, Adam Sander, David E. Schmidt, Maurice Steenhuis, Theo Rispens, Morten A. Nielsen, Benjamin G. Mordmüller, Alexander P.J. Vlaar, C. Ellen van der Schoot, Ramon Roozendaal, Manfred Wuhrer, Gestur Vidarsson. Comparative analysis of spike-specific IgG Fc glycoprofiles elicited by adenoviral, mRNA, and protein-based SARS-CoV-2 vaccines. *iScience*. 2023 September 15.

14. Joseph D. Challenger*, Stijn W. van Beek*, Rob ter Heine, Saskia C. van der Boor, Giovanni D. Charles, **Merel J. Smit**, Chris Ockenhouse, John J. Aponte, Matthew B. B. McCall, Matthijs M. Jore, Thomas S. Churcher†, and Teun Bousema†. Modeling the impact of a highly potent *Plasmodium falciparum* transmission-blocking monoclonal antibody in areas of seasonal malaria transmission. *The Journal of Infectious Diseases*. 2023 July 15.
15. Leen N. Vanheer, Almahamoudou Mahamar, Emilia Manko, Sidi M. Niambele, Koualy Sanogo, Ahamadou Youssouf, Adama Dembele, Makonon Diallo, Seydina O. Maguiraga, Jody Phelan, Ashley Osborne, Anton Spadar, **Merel J. Smit**, Teun Bousema, Chris Drakeley, Taane G. Clark, William Stone , Alassane Dicko, Susana Campino. Genome-wide genetic variation and molecular surveillance of drug resistance in *Plasmodium falciparum* isolates from asymptomatic individuals in Ouélessébougou, Mali. *Scientific reports*. 2023 June 12.

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Merel


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