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Summary

Background Cabamiquine is a novel antimalarial that inhibits *Plasmodium falciparum* translation elongation factor 2. We investigated the causal chemoprophylactic activity and dose–exposure–response relationship of single oral doses of cabamiquine following the direct venous inoculation (DVI) of *P falciparum* sporozoites in malaria-naive, healthy volunteers.

Methods This was a phase 1b, randomised, double-blind, placebo-controlled, adaptive, dose-finding, single-centre study performed in Leiden, Netherlands. Malaria-naive, healthy adults aged 18–45 years were divided into five cohorts and randomly assigned (3:1) to receive cabamiquine or placebo. Randomisation was done by an independent statistician using codes in a permuted block schedule with a block size of four. Participants, investigators, and study personnel were masked to treatment allocation. A single, oral dose regimen of cabamiquine (200, 100, 80, 60, or 30 mg) or matching placebo was administered either at 2 h (early liver-stage) or 96 h (late liver-stage) after DVI. The primary endpoints based on a per-protocol analysis set were the number of participants who developed parasitaemia within 28 days of DVI, time to parasitaemia, number of participants with documented parasite blood-stage growth, clinical symptoms of malaria, and exposure–efficacy modelling. The impact of cabamiquine on liver stages was evaluated indirectly by the appearance of parasitaemia in the blood. The Clopper–Pearson CI (nominal 95%) was used to express the protection rate. The secondary outcomes were safety and tolerability, assessed in those who had received DVI and were administered one dose of the study intervention. The trial was prospectively registered on ClinicalTrials.gov (NCT04250363).

Findings Between Feb 17, 2020 and April 29, 2021, 39 healthy participants were enrolled (early liver-stage: 30 mg [n=3], 60 mg [n=6], 80 mg [n=6], 100 mg [n=3], 200 mg [n=3], pooled placebo [n=6]; late liver-stage: 60 mg [n=3], 100 mg [n=3], 200 mg [n=3], pooled placebo [n=3]). A dose-dependent causal chemoprophylactic effect was observed, with four (67%) of six participants in the 60 mg, five (83%) of six participants in the 80 mg, and all three participants in the 100 and 200 mg cabamiquine dose groups protected from parasitaemia up to study day 28, whereas all participants in the pooled placebo and 30 mg cabamiquine dose group developed parasitaemia. A single, oral dose of 100 mg cabamiquine or higher provided 100% protection against parasitaemia when administered during early or late liver-stage malaria. The median time to parasitaemia in those with early liver-stage malaria was prolonged to 15, 22, and 24 days for the 30, 60, and 80 mg dose of cabamiquine, respectively, compared with 10 days for the pooled placebo. All participants with positive parasitaemia showed documented blood-stage parasite growth, apart from one participant in the pooled placebo group and one participant in the 30 mg cabamiquine group. Most participants did not exhibit any malaria symptoms in both the early and late liver-stage groups, and those reported were mild in severity. A positive dose–exposure–efficacy relationship was established across exposure metrics. The median maximum concentration time was 1–6 h, with a secondary peak observed between 6 h and 12 h in all cabamiquine dose groups (early liver-stage). All cabamiquine doses were safe and well tolerated. Overall, 26 (96%) of 27 participants in the early liver-stage group and ten (83·3%) of 12 participants in the late liver-stage group reported at least one treatment-emergent adverse event (TEAE) with cabamiquine or placebo. Most TEAEs were of mild severity, transient, and resolved without sequelae. The most frequently reported cabamiquine-related TEAE was headache. No dose-related trends were observed in the incidence, severity, or causality of TEAEs.

Interpretation The results from this study show that cabamiquine has a dose-dependent causal chemoprophylactic activity. Together with previously demonstrated activity against the blood stages combined with a half-life of more than 150 h, these results indicate that cabamiquine could be developed as a single-dose monthly regimen for malaria prevention.

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Introduction

Malaria is one of the leading contributors to the overall global burden of disease, with a considerable impact on health-care systems in developing countries.[1,2] WHO estimated 247 million malaria infections in 2021, leading to 619 000 deaths, mostly among children younger than 5 years.[3] Several strategies towards the global eradication of malaria have been recently defined by WHO guidelines, including case management, chemoprevention, and interventions in the final phase of elimination and prevention of re-establishment. Malaria case management consists of prompt effective treatment of uncomplicated malaria to cure the infection as rapidly as possible and to prevent the progression to severe disease. Chemoprevention and chemoprophylaxis use antimalarials as preventive therapy against malaria infection and disease. Chemoprevention involves full therapeutic courses of antimalarials at pre-specified periods, irrespective of infection status, whereas chemoprophylaxis is primarily used by non-immune travellers to malaria-endemic regions.[4] Artemisinin-based combinations are the current standard of care for case management and widely and successfully deployed in all endemic countries. However, there is established resistance against artemisinin in southeast Asia and a first report of resistance in Africa.[5–8]

For chemoprevention, the standard of care is sulfadoxine–pyrimethamine by itself or combined with amodiaquine given monthly during the period at risk. Both drugs have been shown to reduce the number of symptomatic and severe malaria cases by up to 80% in the region where they were used.[9] However, there are increasing reports of resistance against these two drug combinations; in addition, these drugs have other limitations of use, especially in pregnant women (one of the populations targeted by chemopreventive intervention).[10] Chemoprophylaxis, using mainly atovaquone or proguanil and mefloquine, is primarily used by non-immune people travelling to malaria-endemic areas.[4] To sustain and accelerate the effort on malaria elimination, there is a clear need for new interventions, including new antimalarial drugs that could overcome these limitations and provide innovative treatment options.

Cabamiquine is a novel antimalarial drug candidate that inhibits *Plasmodium falciparum* translation elongation factor 2 (*Pf*eEF2). *Pf*eEF2 promotes the guanosine triphosphate-dependent translocation of ribosomes along mRNA during parasite protein synthesis.[11] The potent and selective inhibition of *Pf*eEF2 by cabamiquine inhibits protein synthesis and growth throughout different stages of the parasite's lifecycle, including the initial intrahepatic and subsequent blood stages.[11] The antimalarial activity of cabamiquine has been confirmed in pre-clinical studies using clinical isolates and drug-resistant parasite strains.[12] An ascending dose study showed that cabamiquine was safe and well tolerated and that it effectively cured

Research in context

Evidence before this study

Cabamiquine inhibits *Plasmodium falciparum* translation elongation factor 2, an enzyme essential for protein synthesis in the parasite. We searched PubMed for studies published in English between Jan 1, 2010, and Sept 15, 2022, using the terms "plasmodium translation elongation factor 2 inhibitor", "M5717", and "DDD107498". Pre-clinical data from in-vitro and in-vivo studies show that cabamiquine is active against *P falciparum* at different stages of the lifecycle and, therefore, could be used for chemoprophylaxis. A previous phase 1 clinical trial that characterised the safety, tolerability, pharmacokinetics, and antimalarial activity of cabamiquine in healthy volunteers found that cabamiquine is safe and well tolerated at doses that clear blood-stage *P falciparum*. In addition, the long half-life of cabamiquine indicates its potential for single-dose administration. For the first time in malaria drug development, pharmacokinetic and pharmacodynamic data from in-vitro studies, combined with population pharmacokinetic modelling and simulation, were used to define the pre-erythrocytic activity of a drug in vivo in a clinical trial of a controlled human malaria infection (CHMI) model with an adaptive dose design. The search was updated to February, 2023, and no further studies were found. Altogether, these findings support the investigation of cabamiquine as a single oral-dose drug to assess its chemoprophylactic activity and dose–exposure–response relationship in a controlled *P falciparum* sporozoite infection model of early and late liver-stage malaria induced in healthy volunteers.

Added value of this study

This is the first clinical study to assess the chemoprophylactic potential of cabamiquine using a CHMI model. The data obtained in this study are pivotal for the dose selection of cabamiquine in subsequent trials in conjunction with pharmacometric modelling and simulation approaches.

Implications of all the available evidence

Our study showed that a single oral dose of 100 mg cabamiquine or higher provided 100% protection against both early and late liver-stage malaria. Further assessment of cabamiquine activity against naturally acquired infections, either alone or as part of a combination therapy, might be required to further establish efficacy and minimise antimalarial drug resistance.

P falciparum blood-stage malaria in a controlled human malaria infection (CHMI) model.[13]

Cabamiquine exhibits multi-stage antiplasmodial activity and a long half-life (106–193 h).[13] Therefore, we hypothesised that a single-dose regimen of cabamiquine could provide effective causal prophylaxis against *P falciparum*. The use of CHMI could help to rapidly assess the dose–response relationship and preliminary efficacy as well as accelerate the clinical development of antimalarial agents by enabling informed decisions on dose selection. In this Article, we present the results of a randomised clinical trial assessing the causal chemoprophylactic activity and dose–exposure–response relationship of single oral doses of cabamiquine in a controlled *P falciparum* sporozoite (*Pf*SPZ) challenge infection model of early and late liver-stage malarial infection induced in healthy volunteers, to evaluate the safety and tolerability, and to establish the rate of drug resistance following cabamiquine administration.

Methods

Study design

This phase 1b, randomised, double-blind, placebo-controlled, adaptive, dose-finding, single-centre study was conducted from Feb 17, 2020 (first participant consented) to June 7, 2021 (last participant's last visit), at Leiden University Medical Center, in collaboration with the Centre of Human Drug Research, in Leiden, Netherlands. The independent medical ethics committee Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, Netherlands) approved the study before the start of any study procedures. The study was conducted in accordance with the Declaration of Helsinki, the International Council on Harmonisation guidelines for Good Clinical Practice, and applicable laws and regulations in the Netherlands.

Participants

Male and female participants aged 18–45 years were eligible if they were overtly healthy, as determined by medical screening, and were malaria-naive (no medical history of malaria or possible exposure to malarial parasites). Sex data were collected by self-report; the options available were male, female, or undifferentiated. Participants were split into cohorts; the first cohort was completed just before the onset of the COVID-19 pandemic.[14] Because of the effects of the pandemic, activities were temporarily halted on March 10, 2020. To mitigate the risk of malaria and COVID-19 co-infection in participants and to protect trial staff members, a protocol amendment was made to only include participants who tested negative for SARS-CoV-2 via PCR (appendix p 7). All participants provided written informed consent before trial procedures started.

See Online for appendix

Randomisation and masking

The study participants were randomly assigned (3:1) to the active (cabamiquine) group or the placebo group using an interactive web response system. An independent statistician generated randomisation codes using SAS for Windows (version 9.4) in a permuted block schedule with a block size of four. Participants, investigators, and study personnel were masked to treatment allocation throughout the study. Interim analyses were conducted by a dedicated non-masked biostatistician and pharmacometrician and presented in a masked manner to the safety monitoring committee.

Following a screening period (up to 28 days in length), eligible participants were included across five cohorts with different dose levels and were inoculated with *Pf*SPZ. The study drug cabamiquine or a placebo was administered 2 h after challenge inoculation to investigate the effect on early liver-stage malaria (appendix p 3). Different doses of cabamiquine were explored in the early liver-stage model in the four subsequent cohorts. In between each consecutive cohort, an interim analysis was performed that included an evaluation of participants' safety, and pharmacokinetic and efficacy (cured or not cured status) analyses. The safety monitoring committee established the dose levels for the subsequent cohorts. Briefly, cohorts were either treated with the same dose level or doses were escalated or de-escalated according to the preceding results. The cohorts comprised a minimum of four and a maximum of 12 participants who received a single dose of the study drug or placebo. Safety monitoring committee guidance on dose selection and modification followed a per-protocol adaptive algorithm (appendix p 2). Once the effective dose for early liver-stage malaria had been identified (in cohorts 1–4), the selected doses of cabamiquine were administered 4 days after the *Pf*SPZ challenge to explore their efficacy against late liver-stage malaria infections (in cohort 5; appendix p 4).

Procedures

Participants were inoculated with approximately 3200 purified, aseptic, cryopreserved *Pf*SPZ (15 000/20 µL per vial) of the strain NF54 (*Pf*SPZ challenge; Sanaria, Rockville, MD, USA) via direct venous inoculation (DVI)[15,16] before receiving the study drug or placebo. The study intervention comprised a single, oral dose of cabamiquine or a matching placebo administered either 2 h (early liver-stage group, cohorts 1–4) or 96 h (late liver-stage group, cohort 5) after *Pf*SPZ inoculation. Cabamiquine was supplied as capsules containing 30 or 100 mg of cabamiquine free base. The specified number of cabamiquine or matching placebo capsules were opened, and their contents were dispensed as a water suspension. Study treatments were indistinguishable. Participants fasted for 8 h before and 4 h after cabamiquine administration to mitigate any effects of food on bioavailability. A starting dose of 200 mg cabamiquine was selected on the basis of a population pharmacokinetic model using data from both the first-in-human single ascending dose study[17] and pre-clinical studies. In-vivo

studies in mice have suggested that, for full causal prophylaxis in humans, an area under the concentration–time curve between 0 h and 24 h post-dose ($AUC_{0–24}$) of more than 530 ng·h/mL (corresponding to 3 times the inhibitory concentration 99 [IC_{99}]) is required. Population pharmacokinetic modelling and simulations based on data from the phase 1 study[17] suggested that a dose of 200 mg would exceed the target AUC in all the simulated subjects and hence lead to full prophylaxis.

Using this model, we hypothesised that a dose of 200 mg would prevent liver-stage infection in 50% of the participants. Subsequent dose decisions were based on exposure and cure status. The following dose levels were investigated: 200 mg (starting dose, cohort 1); 100 mg (cohort 2); 30 and 60 mg (cohort 3); and 60 and 80 mg (cohort 4) in the early liver-stage group, and 60, 100, and 200 mg (cohort 5) in the late liver-stage group.

Primary antimalarial rescue treatment consisted of four tablets daily (each with 250 mg atovaquone plus 100 mg proguanil) for 3 consecutive days, which was available to all participants. To ensure participant safety and ensure cure after CHMI, rescue medication was administered either immediately after the confirmation of parasite growth or 28 days after the study intervention if no parasite growth was observed. In addition, artemether–lumefantrine was kept in stock at the study site for use as backup treatment. In the event or suspicion of severe malaria, artesunate was also available for intravenous use.

Outcomes

Study objectives

The primary objective of the study was to assess the causal chemoprophylactic activity and dose–exposure–response relationship of single, oral doses of cabamiquine administered to malaria-naive healthy participants after DVI of a *Pf*SPZ challenge. The secondary objective was to evaluate the safety, tolerability, and pharmacokinetic profile of single, oral doses of cabamiquine in healthy participants following the *Pf*SPZ challenge. The exploratory objective was to determine the rate of drug resistance following cabamiquine administration.

The primary outcomes were (1) the number of participants who developed parasitaemia within 28 days of *Pf*SPZ inoculation; (2) the time to parasitaemia; (3) the number of participants with documented parasite blood-stage growth; (4) clinical symptoms of malaria based on the malaria clinical score;[18] and (5) exposure–efficacy modelling using selected pharmacokinetic (eg, $AUC_{0–24}$, $AUC_{0–168}$, cabamiquine concentration at 24 h post-dose [C_{24}], or C_{168}) and efficacy (cured or not cured) endpoints. The secondary outcomes were (1) monitoring of adverse events, treatment-emergent adverse events (TEAE), or serious adverse events and determination of their association with the study intervention; (2) the incidence of clinically significant changes and abnormalities in safety laboratory parameters (haematology, coagulation, biochemistry, and urinalysis), vital signs (temperature, blood pressure, and pulse rate), and 12-lead electrocardiograms (ECGs); and (3) concentration–time curves and selected pharmacokinetic parameters ($AUC_{0–\infty}$, $AUC_{0–t}$, C_{max}, time to reach maximum blood concentration [t_{max}], apparent terminal half-life [$t_{½}$], total body clearance of drug from blood following oral administration [CL/F], and apparent volume of distribution during the terminal phase following extravascular administration [Vz/F]). The exploratory objective was to determine the incidence of resistance generation following cabamiquine administration via genotyping.

Parasitaemia determination

Parasitaemia was monitored ambulatorily by quantitative PCR (qPCR)[19] before parasite inoculation and then once daily until day 20 after the sporozoite challenge (appendix p 3). If parasitaemia was not detected, this procedure was continued every second day until day 28. Parasitaemia was defined as 100 or more asexual blood-stage parasites per mL. An increase in the parasite number above the positivity threshold of 100 per mL within 28 days of *Pf*SPZ DVI compared with the first parasitaemia measurement was defined as asexual blood-stage parasite growth. Of note, *P falciparum* parasites typically require 6–7 days of liver stage development before initiating asexual blood-stage infection. Upon detection of parasitaemia, blood sampling for qPCR was increased to twice a day until blood-stage parasite growth was observed, after which rescue treatment was initiated. Subsequently, participants with parasitaemia were monitored via daily qPCR assessments until they tested negative for 48 consecutive hours. A final qPCR was performed at the end-of-study visit on day 33 (treatment of early liver-stages) or day 36 (treatment of late liver-stages).

Clinical symptoms of malaria

The validated malaria clinical score[18] indicates the severity of induced malaria infection and uses 14 commonly associated signs and symptoms of malaria, graded on a 3-point scale (0=absent; 1=mild; 2=moderate; and 3=severe), adding up to a maximum possible score of 42. The malaria clinical score was assessed at any timepoint when malaria-related symptoms were observed in participants returning for outpatient qPCR tests.

Dose–exposure–efficacy analysis

Parasitaemia protection has been defined as a binary outcome. Participants with a first positive qPCR outcome (≥100 asexual blood-stage parasites per mL of blood) within 28 days of the *Pf*SPZ challenge were considered not cured; participants who remained negative by qPCR throughout the study period (ie, until day 28) were considered cured. We evaluated the potential relationships between cure and the metrics of cabamiquine exposure using logistic regression. The evaluated exposure metrics included $AUC_{0–24}$, $AUC_{0–168}$, $AUC_{0–\infty}$, C_{24}, and C_{168}.

P falciparum genotyping

In case of parasitaemia and before the initiation of rescue medication, a blood sample was collected to detect possible mutations in recrudescent parasites. Genotyping of the parasite was performed for all participants with observed parasitaemia. The cabamiquine target gene for *Pf*eEF2 was PCR-amplified from all DNA samples using a nested PCR amplification approach. Sanger sequencing of parasite DNA was performed (appendix p 10).

Safety assessments

Safety was assessed through vital sign measurements (temperature, blood pressure, and pulse rate), physical examinations, 12-lead ECGs, blood chemistry and haematology tests, urinalysis, and monitoring of TEAEs related to the study intervention.

Statistical analysis

The study was designed to include a maximum of 50 participants receiving the *Pf*SPZ challenge. Considering its exploratory nature, the sample size was not based on power calculations. Conventionally, previous *Pf*SPZ challenge studies characterised the causal chemoprophylactic activity of antimalarials in 8–12 participants.[20,21] As the exact dose and number of participants showing recrudescence were unknown, the study implemented an adaptive design to change the dose or to expand the dose-level groups on the basis of findings in the previous cohort (a block size of

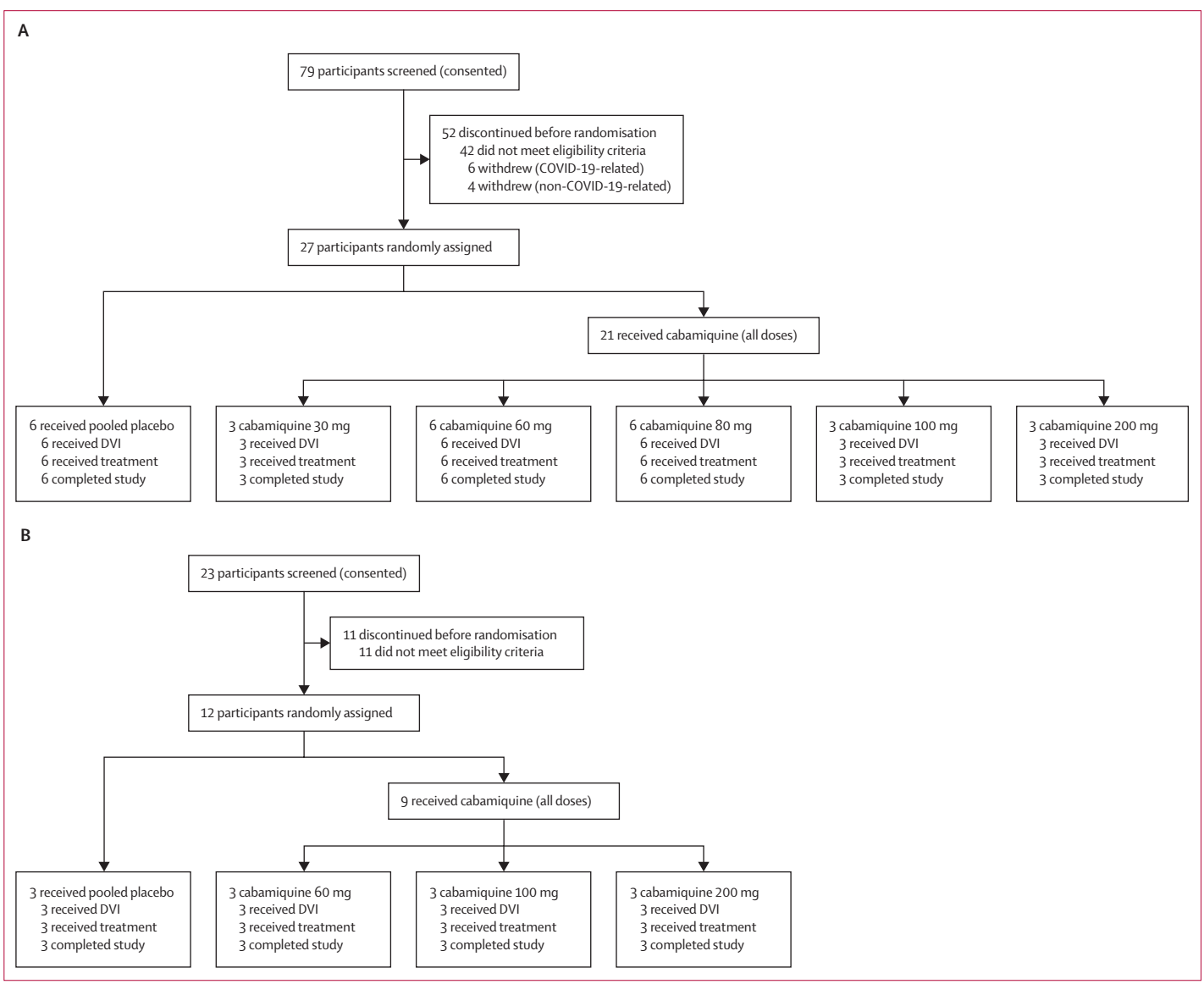

Figure 1: **Trial cohort profile**

(A) Early liver-stage: participant disposition in cohorts 1–4. (B) Late liver-stage: participant disposition in cohort 5. DVI=direct venous inoculation.

four participants; three receiving the intervention and one the placebo). With three actively treated participants, the posterior probability that the true protection rate is 50% or higher was calculated using the Bayesian method. If at least two protected participants out of three actively treated participants are observed in the first cohort, it is likely (probability ≥71%) that the true protection rate is 50% or higher. Hence, in the next cohort, the cohort will be expanded to identify the dose for a 50% protection rate.

All efficacy analyses were performed on a per-protocol analysis set. Causal chemoprophylactic efficacy analyses were performed using descriptive statistics to compare the participants who received cabamiquine with those who received the placebo within the cohorts and in the overall population (pooling all participants who received the placebo across cohorts). The protection rates were defined as 1 minus the proportion of participants with a positive parasitaemia qPCR outcome (≥100 asexual blood-stage parasites per mL of blood) within 28 days of receiving the *Pf*SPZ challenge. The Clopper–Pearson CIs (nominal 95%) for the protection rates were provided. Kaplan–Meier curves and median estimates (with 95% CI) of the time from the DVI of *Pf*SPZ to positive parasitaemia were generated. For exposure–response modelling, the influence of pharmacokinetic exposure metrics on response was assessed using logistic regression.

Safety analyses were performed on the safety analysis set, which was defined as all participants randomised to the study intervention who had received *Pf*SPZ DVI and were administered one dose of the study intervention (cabamiquine or placebo). Safety endpoints were summarised using descriptive statistics. Data from all randomised participants were included for analysis.

We used R, SAS for Windows (version 9.4 or higher), and GraphPad Prism for Windows (version 9.0). Non-compartmental pharmacokinetic analysis was performed using Phoenix WinNonlin (version 6.4 or higher). The trial was prospectively registered on ClinicalTrials.gov (NCT04250363).

Role of the funding source

Authors employed by the healthcare business of Merck KGaA, Darmstadt, Germany, were involved in data acquisition, protocol development, study oversight, data analysis, and data interpretation. The healthcare business of Merck KGaA, Darmstadt, Germany is the sponsor of this phase 1b trial and is currently pursuing the clinical development of cabamiquine. The trial sponsor designed and conceptualised the study with input from all authors.

Results

Between Feb 17, 2020, and April 29, 2021, 39 eligible healthy participants were randomly assigned into five sequential cohorts (figure 1). No protocol deviations were reported. The early liver-stage cohorts 1–4 included

	Part one: early liver-stage group treatment								Part two: late liver-stage group treatment					
	Pooled placebo (n=6)	30 mg (n=3)	60 mg (n=6)	80 mg (n=6)	100 mg (n=3)	200 mg (n=3)	All dose groups (n=21)	Overall (n=27)	Pooled placebo (n=3)	60 mg (n=3)	100 mg (n=3)	200 mg (n=3)	All dose groups (n=9)	Overall (n=12)
Median age, years	24·5	22·0	26·0	22·0	24·0	24·0	24·0	24·0	21·0	24·0	27·0	26·0	26·0	25·0
Sex, n (%)														
Female	0	0	2 (33%)	3 (50%)	0	0	5 (24%)	5 (19%)	2 (67%)	1 (33%)	2 (67%)	1 (33%)	4 (44%)	6 (50%)
Male	6 (100%)	3 (100%)	4 (67%)	3 (50%)	3 (100%)	3 (100%)	16 (76%)	22 (81%)	1 (33%)	2 (67%)	1 (33%)	2 (67%)	5 (56%)	6 (50%)
Ethnicity, n (%)														
Asian	1 (17%)	0	0	0	0	0	0	1 (4%)	0	0	0	0	0	0
Black	0	0	0	0	0	1 (33%)	1 (5%)	1 (4%)	0	1 (33%)	0	0	1 (11%)	1 (8%)
White	5 (83%)	3 (100%)	6 (100%)	5 (83%)	3 (100%)	2 (67%)	19 (90%)	24 (89%)	3 (100%)	2 (67%)	3 (100%)	3 (100%)	8 (89%)	11 (92%)
Mixed	0	0	0	1 (17%)	0	0	1 (5%)	1 (4%)	0	0	0	0	0	0
BMI range (min–max), kg/m^2	20·2–25·3	22·4–23·5	21·7–26·4	22·3–25·8	23·0–25·3	21·7–24·2	21·7–26·4	20·2–26·4	19·7–21·1	22·2–26·0	19·8–23·1	19·8–22·1	19·8–26·0	19·7–26·0

Data are n (%), unless otherwise stated. N is the total number of patients under study; n is a sample of patients under study.

Table 1: **Demographic and baseline characteristics of participants by dose cohort in early and late liver-stage malaria**

	Part one: early liver-stage group treatment						Part two: late liver-stage group treatment			
	Pooled placebo (n=6)	30 mg (n=3)	60 mg (n=6)	80 mg (n=6)	100 mg (n=3)	200 mg (n=3)	Pooled placebo (n=3)	60 mg (n=3)	100 mg (n=3)	200 mg (n=3)
Participants with no parasitaemia within 28 days of PfSPZ challenge	0	0	4 (67%)	5 (83%)	3 (100%)	3 (100%)	0	3 (100%)	3 (100%)	3 (100%)
Median time to parasitaemia in positively tested participants, days (range)*	10 (9–12)	15 (15–18)	22 (20–24)	24	ND	ND	10 (9–11)	ND	ND	ND
Participants over time with positive parasitaemia†	6 (100%)	3 (100%)	2 (33%)	1 (17%)	0	0	3 (100%)	0	0	0
95% CI‡	0·54–1·00	0·29–1·00	0·04–0·78	0–0·64	0–0·71	0–0·71	0·29–1·00	0–0·71	0–0·71	0–0·71
Participants with documented blood-stage parasite growth§	5 (83%)	2 (67%)	2 (33%)	1 (17%)	0	0	3 (100%)	0	0	0
95% CI¶	0·36–1·00	0·09–0·99	0·04–0·78	0–0·64	0–0·71	0–0·71	0·29–1·00	0–0·71	0–0·71	0–0·71

DVI=direct venous inoculation. ND=not determined. qPCR=quantitative PCR. PfSPZ=*Plasmodium falciparum* sporozoites. *The time to first positive parasitaemia was defined as the time (ie, number of days) from the PfSPZ DVI (ie, date of DVI PfSPZ) to the first qPCR outcome of at least 100 asexual parasites per mL of blood. †The number of participants over time with positive parasitaemia was defined as first positive qPCR outcome of at least 100 asexual parasites per mL of blood within 28 days of PfSPZ challenge. ‡The 95% CI of the proportion of participants with positive parasitaemia was based on the Clopper–Pearson method. §Documented blood-stage parasite growth was defined as an increase of qPCR-measured asexual parasites per mL compared with the first parasitaemia measurement (ie, date of DVI PfSPZ). ¶The 95% CI of the proportion of participants with documented blood-stage parasite growth was based on the Clopper–Pearson method.

Table 2: **Causal chemoprophylactic endpoints**

27 participants in a dose de-escalating and staggered cohort enrolment approach. The late liver-stage cohort 5 included 12 participants (table 1).

In the first cohort, treatment with 200 mg cabamiquine showed no parasitaemia in three of four participants, with the fourth participant receiving the placebo. In the second cohort (four participants), the dose was reduced to 100 mg as per the protocol. The outcome of the 100 mg cabamiquine or placebo dose also showed no parasitaemia in three participants receiving cabamiquine, with the fourth participant receiving the placebo.

To further identify the lowest, fully causal prophylactic dose, in the next cohort (cohort 3), 60 mg and 30 mg doses were tested. In the 30 mg group in this cohort, all four participants (including one who received the placebo) developed parasitaemia, whereas in the 60 mg group, the one participant who received the placebo and one of three participants who received cabamiquine developed parasitaemia. These six participants had an AUC_{0-24} below the IC_{99} of 180 ng·h/mL. This observation suggested that the initial target concentration, which was 3 times higher than the IC_{99}, was higher than needed and $1\times IC_{99}$ might be more appropriate. Therefore, in cohort 4, three of four participants were administered 60 mg cabamiquine and one received the placebo. In the remaining two groups of four participants, six participants were treated with 80 mg cabamiquine and two received the placebo. This improved the exploration of the inflection point that correlated with causal prophylactic efficacy. None of the participants in the 60 mg treatment group in this cohort developed parasitaemia, whereas five of eight participants in the 80 mg cabamiquine or placebo groups did not develop parasitaemia. In cohort 5, three doses of cabamiquine (200, 100, and 60 mg) were evaluated with four participants per group. All doses prevented the development of late liver-stage parasitaemia (table 2).

Cabamiquine prevented parasitaemia within 28 days after the *Pf*SPZ challenge in 100% of participants who were administered 200 mg and 100 mg in both the early and late liver-stages (table 2). The median time to parasitaemia in early liver-stage malaria was prolonged to 15, 22, and 24 days in participants who received 30, 60, and 80 mg doses of cabamiquine, respectively, compared with 10 days for the pooled placebo (figure 2). The Kaplan–Meier curves for the early liver-stage group showed that the time to positive parasitaemia was dose-dependent and significantly longer for participants who received cabamiquine compared with those who received placebo (figure 3A). Administration of cabamiquine during late liver-stage infection resulted in protection from parasitaemia, whereas all participants who received placebo developed parasitaemia (figure 3B). All the participants with positive parasitaemia showed documented blood-stage parasite growth, except one participant in the pooled placebo group and one participant in the 30 mg cabamiquine group. Both of these participants had high blood-stage parasite counts (1757 and 1461) and were therefore directly treated with rescue medication. Consequently, the second qPCR

assessment was lower than the first (table 2), and the participants thus did not fulfil the definition of blood-stage parasite growth.

Most participants did not exhibit any malaria symptoms in both the early and late liver-stage treatment regimens, and those reported were mild in severity. The peak malaria clinical score recorded was 7/42 at day 18 for a participant who received cabamiquine at a concentration of 30 mg (n=1) and 6/42 at day 14 for three people who received the placebo.

The associations between cure and metrics of cabamiquine exposure as well as between liver-stage groups (early *vs* late) showed causal prophylactic activity with a significant ordered association between dose exposure and parasitaemia prevention (appendix pp 5–6). Systemic cabamiquine concentrations increased with the increase in the oral cabamiquine dose administered across the groups. The median maximum concentration time was found to be 1–6 h, with a secondary peak observed between 6 h and 12 h in all cabamiquine dose groups.

Cabamiquine exhibited a long half-life (ranges 121–494 h at doses ≥60 mg) and greater than a dose-proportional increase in systemic exposure. The summary statistics of pharmacokinetic parameters according to cabamiquine dose level for early and late liver-stage parasitaemia are presented in the appendix (pp 12–14). These results are in line with previous data on cabamiquine.[13] Parasitaemia prevention as a function of exposure was explored by applying logistic regression models to the data, using AUC_{0-24}, AUC_{0-168}, $AUC_{0-\infty}$, C_{24}, and C_{168} as predictors. All exposure metrics predicted parasitaemia prevention equally well (appendix p 12). Genotyping of *P falciparum* from all participants who developed breakthrough parasitaemia did not reveal any mutations in the gene encoding *Pf*eEF2.

Overall, 26 (96%) of 27 participants in the early liver-stage group and ten (83%) of 12 participants in the late liver-stage group reported at least one TEAE with cabamiquine or placebo (table 3). The incidences of all TEAEs reported during the study were similar between the cabamiquine dose groups and the pooled placebo group. Most TEAEs reported were of mild severity and transient and they resolved without sequelae. No apparent difference in severity was observed between the cabamiquine and placebo groups (data not shown). Of 208 TEAEs, three were classified as severe: two in the early liver-stage group (experienced by a single participant) and one in the late liver-stage group. All three TEAEs were related to the rescue medication and consisted of nausea (n=2) and vomiting (n=1). The incidence of TEAEs related to the study intervention in the early liver-stage group was two (33%) of six in the placebo group and eight (38%) of 21 in the cabamiquine group. In the late liver-stage group, the incidence was 0 in the placebo group and one (11%) of nine in the cabamiquine group. Of 208 TEAEs reported, 16 (8%) were found to be related to cabamiquine or placebo administration (11 TEAEs in the cabamiquine group, three in the placebo group; two TEAEs were related to the

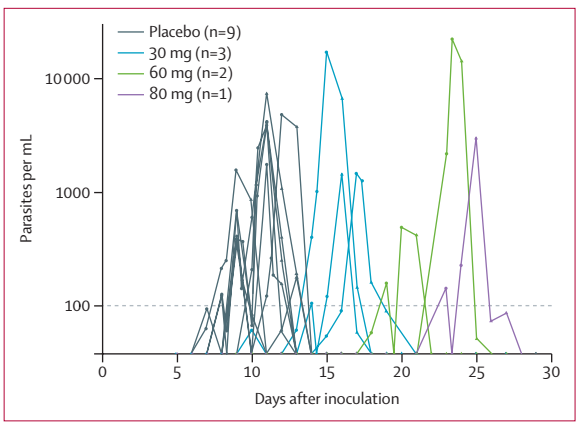

Figure 2: **Blood-stage parasite concentrations versus time in participants who received placebo or developed parasitaemia**
n is the number of participants who developed parasitaemia. The filled circles represent datapoints pre-rescue medication and triangles represent datapoints post-rescue medication.

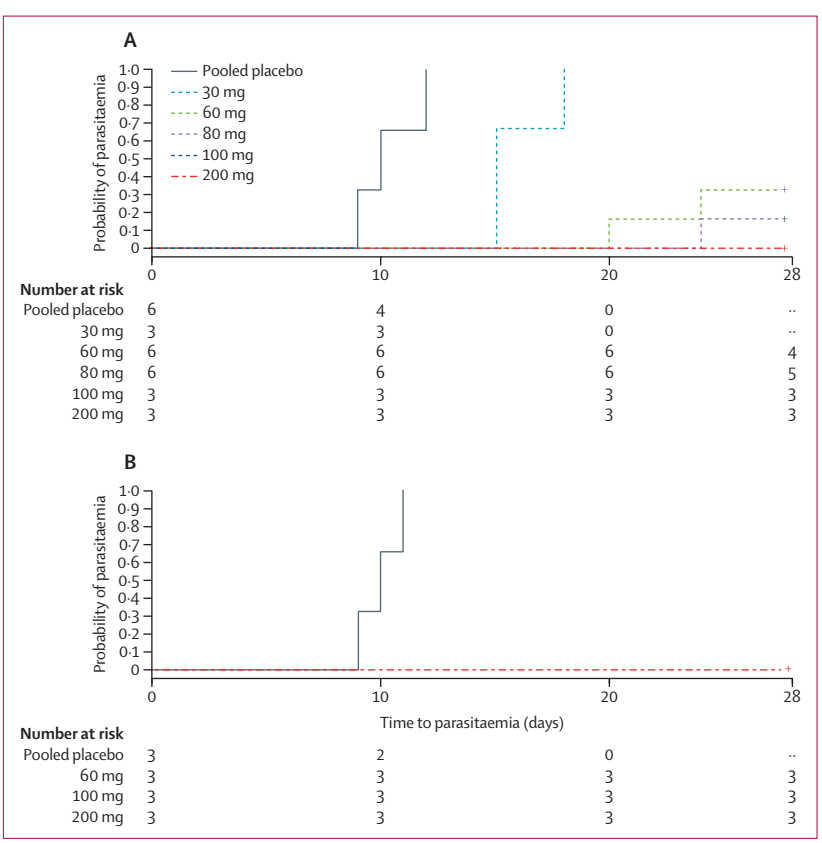

Figure 3: **Kaplan–Meier curve of the probability of blood-stage parasitaemia**
(A) Treatment of early liver-stage malarial infection: time to parasitaemia. Log-rank 30 mg: p=0·0113; 60 mg: p=0·0007; 80 mg: p=0·0007; 100 mg: p=0·0113; 200 mg: p=0·0113 + censored; p values were presented using the log-rank test for each cabamiquine dose versus the pooled placebo. (B) Treatment of late liver-stage malarial infection: time to parasitaemia. Log-rank 60 mg: p=0·0246; 100 mg: p=0·0246; 200 mg: p=0·0246 + censored; p values were presented using the log-rank test for each cabamiquine dose versus the pooled placebo.

	Part one: early liver-stage group								Part two: late liver-stage group					
	Pooled placebo (n=6)	30 mg (n=3)	60 mg (n=6)	80 mg (n=6)	100 mg (n=3)	200 mg (n=3)	All dose groups (n=21)	Overall (n=27)	Pooled placebo (n=3)	60 mg (n=3)	100 mg (n=3)	200 mg (n=3)	All dose groups (n=9)	Overall (n=12)
Any TEAE	5 (83%)	3 (100%)	6 (100%)	6 (100%)	3 (100%)	3 (100%)	21 (100%)	26 (96%)	3 (100%)	3 (100%)	2 (67%)	2 (67%)	7 (78%)	10 (83%)
Any severe TEAE	0	0	0	0	1 (33%)	0	1 (5%)	1 (4%)	0	0	1 (33%)	0	1 (11%)	1 (8%)
Any TEAE related to study treatment	2 (33%)	0	2 (33%)	2 (33%)	1 (33%)	3 (100%)	8 (38%)	10 (37%)	0	1 (33%)	0	0	1 (11%)	1 (8%)
Any serious TEAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Data are presented as the number of participants with at least one adverse event observed after dosing with cabamiquine or placebo (%). The safety analysis set was defined as all participants randomly assigned to the study intervention who had been inoculated via direct venous inoculation of *Plasmodium falciparum* sporozoites and who had been administered one dose of the study intervention (cabamiquine or placebo). TEAE=treatment-emergent adverse event.

Table 3: **Adverse events by dose cohort in early and late liver-stages**

protocol procedure but an association to the study intervention could not be ruled out). The most frequently reported cabamiquine-related TEAE was headache. No serious TEAEs occurred during the study, and none resulted in study discontinuation. No dose-related trends were observed in the incidence, severity, or causality of TEAEs. No clinically significant abnormalities were observed in laboratory parameters, vital signs measurements, or 12-lead ECG readings related to cabamiquine (data not shown). Single oral doses of cabamiquine were found to be safe and well tolerated at all investigated doses.

Discussion

This study evaluated the causal chemoprophylactic activity and dose–exposure–response relationship of cabamiquine in malaria-naive healthy participants using a CHMI model for late and early liver-stage malarial infection. Pre-clinical data suggest that cabamiquine is active against liver-stage and blood-stage *Plasmodium* infections and therefore could be used for chemoprophylaxis.[11,22] In a previous first-in-human study, the novel *Pf*eEF2 inhibitor cabamiquine was well tolerated in healthy participants at 150, 400, and 800 mg doses and showed a dose-dependent effect in curing blood-stage malaria in a *P falciparum* CHMI model.[13]

The present study showed that a single oral dose of 100 mg cabamiquine or higher provided 100% protection in a liver-stage CHMI model. Cabamiquine was active and effective against the early and late liver-stages of *P falciparum* infection in a dose-dependent manner. Single 100 mg and 200 mg doses of cabamiquine provided complete protection when treating both early and late liver-stages. A positive exposure–efficacy relationship was established across exposure metrics in a logistic regression model.

In participants who were not fully protected, a marked delay in parasitaemia occurred in a dose-dependent manner in the 30–80 mg cabamiquine dose range. This delay in parasitaemia is probably due to a decreased exposure to cabamiquine in doses less than 100 mg (effective dose), resulting in static effects on liver-stage parasite growth, but it was ultimately insufficient to prevent the onset of the next phase of blood-stage development. Although the lower doses (30, 60, and 80 mg) showed antimalarial activity, which was apparent as a dose-related delay in the onset of blood-stage parasitaemia, these doses did not have a clear effect on the severity of parasitaemia or on the speed (steepness of curve of parasitaemia over time for individual participants) at which parasitaemia developed. The study adopted a unique design of exploring both early and late liver-stage malarial infection. Interestingly, no signs were noted of impeded chemoprophylaxis for the doses tested, as indicated by a delay in cabamiquine administration, suggesting sufficient cabamiquine exposure and antimalarial activity throughout the first 96 h of sporozoite liver-stage development. Lastly, single oral doses of cabamiquine were found to be safe and well tolerated, and no mutations were observed in recrudescent parasites harvested from patients with breakthrough parasitaemia.

Cabamiquine has a prolonged antimalarial activity that arrests parasite growth by inhibiting protein synthesis, resulting in the reduction of the parasite burden and making it convenient for use as a prophylaxis for travellers. In addition, the requirement of frequent dosing is circumvented owing to the long half-life of cabamiquine (mean half-life values ranged 106–193 h at doses ≤200 mg), enabling potential single-dose use.[13] However, this phase 1b study has some limitations. Given the rationale to determine a dose range for cabamiquine, the study was conducted in malaria-naive healthy adults who were primarily White and from the Netherlands. Thus, the study population represented only a small fraction of the target population and did not comprise different geographies, genetic diversity, a range of age groups, or a vulnerable population. Therefore, the study population differs from the populations at risk of malaria infection in endemic regions. Additional field trials of cabamiquine in endemic regions and with a heterogeneous study population might be required. Another limitation is the inoculation manner that occurred via intravenous injection using a single *Pf*SPZ

strain (NF54; as in previous CHMI studies assessing causal efficacy[21]), which is more stringent than mosquito bite (naturally acquired), for which polyclonal infections can occur under natural conditions. Furthermore, there are no existing biomarkers to follow human malaria liver infection and the effect of the drugs can only be monitored indirectly by assessing delayed or negative blood-stage infection; thus, a potential additional blood-stage effect of the drug cannot be fully excluded.

The interval between drug administration and malaria challenge varies considerably in studies assessing the causal chemoprophylactic activity of antimalarial compounds in CHMIs, with study treatment being administered either before or after the malaria challenge. No consensus has been reached for these kinds of studies. In studies investigating DSM265, the drug was administered 1, 3, or 7 days before the challenge.[20,21] Kublin and colleagues[23] used a CHMI model to evaluate the efficacy of KAF156 administered either 3 h before the challenge or 21 h after the challenge. Recently, Chughlay and colleagues[24] administered P218 in a two-dose regimen at 2 h and 48 h post-DVI, which was similar to the regimen used in our study. The present study was designed to mimic the situation that travellers might encounter when parasite replication starts during the first 24 h (early-stage) of malarial infection. In the first four cohorts, cabamiquine was administered 2 h after DVI so that sporozoites can be deposited in the liver without any interference by cabamiquine and the effect of the study intervention on pre-erythrocytic liver stages can be evaluated. In the subsequent cohort, selected doses of cabamiquine were administered 96 h after DVI to additionally assess the effect of the drug on late liver-stage parasitaemia. Therefore, the chosen study design enabled the assessment of both the early and late stage development of parasitaemia and offers valuable insights on the efficacy of cabamiquine in late liver-stage malaria. Cabamiquine was effective against both liver-stage and blood-stage malarial infection, similar to the causal prophylactic activity of DSM265 against *P falciparum*.[20] To the best of our knowledge, our study is one of only a few that used both an early and late sporozoite CHMI model in combination with an adaptive dosing strategy. As there is a risk of emergence of resistant parasites, especially in multiple dosing regimens or prolonged use, cabamiquine would need to be partnered with another drug. Further assessment of cabamiquine activity against naturally acquired *P falciparum* infections is required, either alone or as a combination therapy with pyronaridine,[25,26] a potential combination partner currently under evaluation.

In conclusion, this phase 1b study showed that cabamiquine exhibits causal chemoprophylactic activity in a dose-dependent manner. A single dose of 100 mg cabamiquine or higher provided 100% protection in a liver-stage CHMI model, thereby supporting patient compliance. These results will guide rational dose selection and dosing interval in future clinical trials. Ultimately, the utility of cabamiquine could pave the way to combination therapy for future use to circumvent antimalarial drug resistance.

Contributors

JLvdP, WMB, ÖY, MR, and AK were responsible for data acquisition and contributed to the study design, data analysis, and data interpretation. VPK, MB, SKD, and VT collected, analysed, and interpreted the data. DB and IMCK were responsible for data collection and contributed to the overall study design and data analysis. AS performed statistical analysis and results interpretation. AT was responsible for managing the safety aspects of the study and verifying underlying data. CO was responsible for defining the overall strategy, aligning the study design with the overall project strategy, providing inputs for result analysis and interpretation, and providing operational support. TS was responsible for the design, analysis, and interpretation of preclinical data. JW and DAF contributed to the analysis and interpretation of data. JPRK, OACL, GVTR, and LCAS were responsible for logistics, safety assessments, data collection, and data entry into the database. RvS, JS, and PM were responsible for the import, quality control, release, storage, and preparation of the *Pf*SPZ challenge product. EW was responsible for data acquisition and the implementation and validation of the malaria quantitive PCR for endpoint assessment in this trial. The trial sponsor (the healthcare business of Merck KGaA, Darmstadt, Germany) designed the study with input from all authors. All authors contributed to data interpretation and reviewed the manuscript. AK, AS, and MR accessed and verified the data. A professional medical writer employed by Merck Specialities in Bengaluru, India, drafted the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

MB, ÖY, AT, AS, and AK are employed by the healthcare business of Merck KGaA, Darmstadt, Germany (the study sponsor). DB is employed by Merck Pty in Modderfontein, South Africa. CO and TS are employed by the Global Health Institute of Merck, Ares Trading in Eysins, Switzerland. WMB is a former (retired) employee of the Merck Institute for Pharmacometrics, Merck Serono in Lausanne, Switzerland. JW received funding for consulting with the healthcare business of Merck during the course of this study. All other authors declare no competing interests.

Data sharing

Any requests for data by qualified scientific and medical researchers for legitimate research purposes will be subject to the healthcare business of Merck KGaA, Darmstadt, Germany's data sharing policy. All requests should be submitted in writing to the healthcare business of Merck's data sharing portal https://www.merckgroup.com/en/research/our-approach-to-research-and-development/healthcare/clinical-trials/commitment-responsible-data-sharing.html. When the healthcare business of Merck has a co-research, co-development, co-marketing, or co-promotion agreement, or when the product has been out-licensed, the responsibility for disclosure might be dependent on the agreement between parties. Under these circumstances, the healthcare business of Merck will endeavour to gain agreement to share data in response to requests.

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