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Characterizing the pharmacological interaction of the antimalarial combination artefenomel-piperaquine in healthy volunteers with induced blood-stage *Plasmodium falciparum* to predict efficacy in patients with malaria

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Abstract

Background The combination antimalarial artefenomel-piperaquine failed to achieve target efficacy in a phase 2b study in Africa and Vietnam. We retrospectively evaluated whether characterizing the pharmacological interaction of this antimalarial combination in a volunteer infection study (VIS) would have enabled prediction of the phase 2b study results.

Methods Twenty-four healthy adults enrolled over three consecutive cohorts were inoculated with *Plasmodium falciparum*-infected erythrocytes on day 0. Participants were randomized within each cohort to one of seven dose combination groups and administered a single oral dose of artefenomel-piperaquine on day 8. Participants received definitive antimalarial treatment with artemether-lumefantrine upon parasite regrowth or on day 42 ± 2. The general pharmacodynamic interaction (GPDI) model implemented in the Bliss Independence additivity criterion was developed to characterize the pharmacological interaction between artefenomel and piperaquine. Simulations based on the model were performed to predict the outcomes of the phase 2b combination study.

Results For a dose of 800 mg artefenomel administered with 640 mg, 960 mg, or 1440 mg piperaquine, the simulated adequate parasitological response at day 28 (APR_{28}), incorporating actual patient pharmacokinetic (PK) data from the phase 2b trial, was 69.4%, 63.9%, and 74.8%, respectively. These results closely matched the observed APR_{28} in the phase 2b trial of 67.0%, 65.5%, and 75.4%, respectively.

Conclusions These results indicate that VIS offer an efficient means for informing antimalarial combination trials conducted in the field, potentially expediting clinical development.

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Keywords Artefenomel, Piperaquine, Antimalarial, Combination, Volunteer infection study, Pharmacokinetics, Pharmacodynamics

Background

Malaria remains a significant threat to global health, with an estimated 249 million cases and 608,000 deaths in 2022 [1]. Artemisinin-based combination therapies (ACTs) are the recommended first-line treatment for uncomplicated *Plasmodium falciparum* malaria, although the emergence of artemisinin-resistant *P. falciparum* parasites in Southeast Asia [2], and more recently in East Africa [3–5], threatens the utility of ACTs and progress toward malaria eradication. The development of novel antimalarial therapies is required to control malaria in regions with high prevalence of artemisinin resistance and to prevent the spread of resistance to areas where ACTs are currently effective.

Combination therapies are the focus of antimalarial drug development, aiming to reduce the risk of selecting resistant mutants and to target multiple stages of the parasite lifecycle including the transmissible gametocytes. Further, using a combination of two or more drugs is typically considered a prerequisite for achieving cure, given that new antimalarial combination treatments will ideally be given as a single dose or as a very short course of therapy. In the case of ACT regimens, all of which require multiple-dose treatments, the artemisinin component rapidly reduces the parasite burden, while the partner drug with a long elimination half-life is relied upon to clear residual parasites. Combination partners in approved ACTs include lumefantrine, amodiaquine, piperaquine, mefloquine, pyronaridine, and sulfadoxine-pyrimethamine [6].

Artefenomel (previously known as OZ439) is an investigational antimalarial that progressed from pre-clinical [7] to phase 1 [8, 9] and phase 2 [10] clinical studies. Artefenomel is an ozonide and is thought to act in a similar manner to the artemisinins by reacting with iron within the parasite food vacuole to produce free radicals, leading to alkylation of key parasite proteins [11]. In patients with malaria, artefenomel exhibits similar antimalarial activity to artemisinins, with parasite clearance half-lives of 4.1 h to 5.6 h following single doses of 200–1200 mg [10]. However, artefenomel has a longer elimination half-life than artemisinins (approximately 50 h for artefenomel [10] compared to less than 10 h for artemisinin derivatives [12]).

Co-administration of artefenomel with piperaquine was considered a potential alternative to current ACTs, leading to testing of this combination in a phase 2b clinical trial undertaken in Africa and Vietnam [13]. Patients with uncomplicated *P. falciparum* malaria (predominantly children ≤ 5 years of age) were administered a single 800 mg dose of artefenomel plus piperaquine in ascending single doses (640 mg, 960 mg, or 1440 mg). Unfortunately, none of the treatment arms reached the target efficacy of > 95% adequate clinical and parasitological response at day 28 ($ACPR_{28}$), with $ACPR_{28}$ of 70.8%, 68.4%, and 78.6% for each dose group, respectively [13]. Single dose combinations that may have achieved target efficacy were retrospectively estimated via simulations using trial data. However, doses of the combination that were predicted to be required to reach this target (800 mg artefenomel plus ≥ 2000 mg piperaquine; 1200 mg artefenomel plus ≥ 960 mg piperaquine; or 1600 mg artefenomel plus ≥ 320 mg piperaquine) were deemed unfeasible due to safety, tolerability and practical considerations [13].

Malaria volunteer infection studies (VIS) using the induced blood-stage malaria (IBSM) model involve the inoculation of healthy, malaria naïve adults with parasites followed by administration of the test antimalarial when a predefined parasitemia threshold has been reached, and have successfully characterized the antimalarial activity of several drugs in monotherapy, including artefenomel [8] and piperaquine [14]. Pharmacokinetic/pharmacodynamic (PK/PD) modeling using data from the previously conducted artefenomel VIS involving a Caucasian volunteer population was found to accurately predict the antimalarial activity of artefenomel monotherapy in a phase 2a trial in Thailand [10]. VIS also have the potential to investigate PK/PD interactions between two or more antimalarials when administered in combination; such a study was conducted for the first time with the IBSM model to investigate artefenomel in combination with another antimalarial candidate DSM265 [15]. Data from this study were subsequently used for PK/PD simulation to predict that a single dose curative dose of 800 mg of artefenomel combined with 450 mg of DSM265 would likely achieve the target ACPR [16].

In the current study, we aimed to evaluate the pharmacological interaction between artefenomel and piperaquine in a VIS to test the hypothesis that such an approach would have predicted the outcome of the combination phase 2b study. Such an evaluation was

considered important given the ethical, expense, time commitment, and safety considerations associated with a failed clinical trial of an antimalarial combination in an endemic setting.

Methods

Study design and participants

This was a randomized, open-label, VIS using the *P. falciparum* IBSM model. Healthy, malaria naïve, adults aged 18–55 years were eligible for inclusion (Additional file 1: Text S1). The study was conducted at Q-Pharm (Brisbane, Australia) following approval by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee. All participants gave written informed consent before enrolment. This study was registered on ClinicalTrials.gov on 11 May 2018 with registration number NCT03542149.

Procedures

The study consisted of three consecutive cohorts of 8 participants per cohort. Participants were inoculated intravenously with *P. falciparum* 3D7-infected human erythrocytes (approximately 2800 viable parasites) on day 0 and parasitemia was monitored by quantitative polymerase chain reaction (qPCR) targeting the gene encoding *P. falciparum* 18S rRNA (lower limit of quantification [LLOQ] 32 parasites/mL whole blood) [17, 18]. Single oral doses of artefenomel and piperaquine phosphate were administered concurrently on day 8 in a fasted state (participants were fasted for 6 h pre- and post-dosing). Artefenomel granules (200 mg or 400 mg) with the excipient α-tocopherol polyethylene glycol 1000 succinate (PCI Pharma Services, UK) were mixed with water to form an oral suspension, and sucrose was added prior to administration to make the oral suspension palatable. After the participant consumed the suspension, the cup was rinsed with water which was then used to facilitate swallowing the piperaquine tablets (160 mg per tablet; PCI Pharma Services, UK). Participants were confined to the clinic for 72 h post-dosing and returned as outpatients for follow-up visits until the end of the study visit on day 45 ± 2. Participants received a standard curative course of artemether-lumefantrine (Riamet®; Novartis Pharmaceuticals Pty Ltd, Australia) upon parasite regrowth (defined as ≥ 5000 parasites/mL and a twofold increase within 48 h) or on day 42 ± 2 if parasite regrowth had not occurred. Asexual parasite regrowth was distinguished from gametocytemia using qRT-PCR targeting gametocyte-specific transcripts [19].

Blood samples were collected pre-dose and at the following time-points after dosing to determine artefenomel and piperaquine plasma concentration: 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72, 96, 168, 240, 336, 504, 672, and 840 h. Drug concentrations were determined using liquid chromatography-tandem mass spectrometry as described previously for artefenomel [9] and piperaquine [20] (LLOQ 1 ng/mL for each). To monitor total parasitemia by 18S qPCR, blood samples were collected before inoculation on day 0, day 4, twice daily on days 5–7, pre-dosing, and 2, 4, 8, 12, 16, 20, 24, 30, 36, and 42 h post-dosing on day 8 and 9, twice daily on days 10–13, and every 1 to 3 days until the end of study (day 45 ± 2). The timing of all study procedures is presented in Additional file 2: Table S1.

Sample size, randomization, and dose selection

The intended sample size (24 participants) was not based on a formal power calculation but, based on previous studies [15], was considered adequate to assess the primary objective of the study. More than one dose level was tested within each cohort to optimally characterize the PK/PD relationship of the combination. Participants were randomized within each cohort to a dose group on the day of dosing. Cohort 1 consisted of 4 dose groups while cohort 2 and cohort 3 consisted of 2 dose groups. Randomization was balanced over the dose groups within each cohort. The randomization schedule was generated using Stata version 15 (StataCorp, College Station, TX, USA). No blinding was performed.

The doses of artefenomel and piperaquine were chosen to facilitate modeling to estimate the PK/PD relationship. For cohort 1, doses were based on the PK/PD relationships observed in IBSM monotherapy studies of artefenomel [8] and piperaquine [14] and the potential PD interaction effects of the combination based on the phase 2b combination trial [13]. The doses for cohorts 2 and 3 were decided based on a preliminary PK/PD model which utilized data from the preceding cohort and from the artefenomel and piperaquine monotherapy VIS. Different dose combinations were evaluated using popED [21] assuming known PK parameters. Estimation errors for the PD parameters and the D-optimality criterion were considered [21]. Promising doses based on this evaluation for the next cohort were simulated and doses selected following discussion between the modelers and study investigators.

Pharmacokinetic/pharmacodynamic modeling

A graphical overview of the PK/PD modeling and simulation approach taken in this study is presented in Additional file 3: Fig. S1. The VIS PK/PD model was built in a stepwise manner, using data from the current study as well as all available parasitemia, drug concentration, and participant demographic data from the artefenomel [8] and piperaquine [14] monotherapy VIS (Additional file 2: Table S2). A population PK model was firstly

developed to obtain individual PK parameter estimates which adequately described the observed individual PK profiles. Drug-drug interaction on the PK parameters was tested as inhibition of the clearance of one drug by the concentrations of the other drug. The PK/PD model was then built using the individual PK parameter estimates as regressors to evaluate the relationship between artefenomel and piperaquine plasma concentration and parasite killing. A PD model for each compound alone was built independently to estimate the single-drug effect parameters. The combined effects of artefenomel and piperaquine were evaluated using the general pharmacodynamic interaction model (GPDI model [22]) implemented in the Bliss Independence additivity criterion. This GPDI model characterizes the type of drug interactions (i.e., synergistic, antagonistic, or asymmetric) and describes the interaction of perpetrator and victim drugs on the maximum effect (E_{max}) and/or potency (concentrations with half-maximal killing effect [EC_{50}]). Model evaluation and selection was guided by visual inspection of goodness of fit plots, of individual PK and PD profiles, plausibility and precision of parameter estimates, and fit statistics such as Bayesian information criterion (BIC).

Simulations of clinical success in patients

To assess the translatability of the VIS PK/PD data, the adequate parasitological response at day 28 (APR_{28}) was predicted based on a PK/PD model and compared with corresponding observations from the phase 2b trial of the artefenomel + piperaquine combination [13]. APR_{28} was defined as a solely parasitemia-based simplification of the $ACPR_{28}$ clinical success criterion [23], in the absence of body temperature data relevant for the evaluation of $ACPR_{28}$ (Additional file 1: Text S2).

Two separate simulations were performed using either the PK model derived from VIS data or actual PK data from patients in the phase 2b trial (in which case the estimated individual patient PK parameters were fixed as regressors). For both simulations, the PD parameters for artefenomel and piperaquine were sampled based on the respective VIS PK/PD monotherapy models, and the PD interaction parameters were sampled based on the VIS GPDI model. The sampling of the VIS PD models and interaction parameters (and the VIS PK model where appropriate) was done in two steps. First, 250 sets of reference population parameter values were sampled from the parameter uncertainty distribution. Then, for each such set, a population of individual parameter sets was sampled from the inter-individual variability distribution. The number of patients in each simulated population matched the population size of the phase 2b trial (Additional file 2: Table S3). Simulations used the actual baseline parasitemia values recorded for patients in the phase 2b trial, and patient body weight was accounted for in simulations using the VIS PK model (Additional file 2: Table S3). Parasites were assumed to grow exponentially in patients at a growth rate of 0.048 h^{-1} (equivalent to a multiplication rate of ~tenfold per 48-h asexual cycle) [24, 25]

The APR_{28} was determined for the observed and simulated parasitemia time courses. For the observed parasitemia, the proportion of patients with APR_{28} status was reported and the 95% CI was estimated using the Clopper-Pearson method. For the simulated parasitemia, the median APR_{28} proportion over the 250 populations was reported and the 0.025th and 0.975th quantiles were used as an approximation of the 95% CI.

All data processing, analysis, model setup, and modeling analysis, including goodness-of-fit plots, was performed in R 3.5.1 using the IQRtools package 1.0.0 (https://iqrtools.intiquan.com). Nonlinear mixed effects (NLME) modeling was performed with Monolix 2018R2 (http://lixoft.com) using Stochastic Approximation Expectation Maximization (SAEM) for parameter estimation. All raw data and code used for PK/PD modeling and simulations are included in Additional File 4 (VIS parasitemia data), Additional File 5 (VIS PK data), Additional File 6 (phase 2b patients baseline parasitemia, weight, age, and PK Bayes estimates), Additional File 7 (phase 2b patients parasitemia data), Additional File 8 (VIS PK/PD model code text file), and Additional File 9 (VIS PK/PD model code MLXTRAN file).

Non-compartmental pharmacokinetic analysis

Non-compartmental PK parameters calculated were: maximum observed concentration (C_{max}), time to reach C_{max} (t_{max}), area under the concentration–time curve (AUC) from time 0 (dosing) to the last sampling time at which the concentration is ≥ the LLOQ ($AUC_{0\text{-last}}$), AUC from time 0 extrapolated to infinity ($AUC_{0\text{-inf}}$), apparent elimination half-life ($t_{½}$), apparent total body clearance (CL/F), apparent total volume of distribution (Vz/F), elapsed time from dosing at which drug concentration was first quantifiable (t_{lag}), and the apparent terminal elimination rate constant (λ_{inf}). Non-compartmental pharmacokinetic analysis was performed in R version 3.5.1 (https://R-project.org) using the IQR tools package 1.0.0.

Parasite clearance analysis

The parasite reduction ratio (PRR) and corresponding parasite clearance half-life ($PCt_{1/2}$) were estimated, with the former expressed as the ratio of the parasite density decrease over 48 h following dosing (PRR_{48}). The PRR_{48} and parasite clearance half-life were estimated using the slope of the optimal fit for the log-linear relationship

of the parasitemia decay as described previously [26]. Briefly, the decay rate (slope coefficient from the log-linear decay regression) for each participant was calculated initially, and then the weighted average slope estimate and corresponding standard error were calculated using an inverse-variance method, which was used to estimate the dose-specific PRR_{48} and 95% CI. The $PCt_{1/2}$ is a transformation of the slope coefficient into a time period. Parasite clearance analyses were performed in R version 3.5.1. The percentage of participants with parasite regrowth following dosing was also calculated.

Safety and tolerability analysis

The incidence, severity, and relationship to artefenomel + piperaquine administration of adverse events (AEs) were monitored. The period of observation for the collection of AEs extended from the time of inoculation with the malaria challenge agent up to the end of the study (Additional File 2: Table S1). AE severity was assessed in accordance with the Common Terminology Criteria for Adverse Events [27] (mild = grade 1; moderate = grade 2; severe = grade 3; life-threatening consequences = grade 4; death related to AE = grade 5). In addition, an AE was classified as a serious adverse event (SAE) if it met one of the following criteria: resulted in death, was life-threatening, required inpatient hospitalization, resulted in persistent or significant disability, was a congenital anomaly, was considered medically important, constitutes a possible Hy's law case. The investigator assessed if AEs were related to artefenomel + piperaquine and/or to the malaria challenge (unrelated, unlikely, possible, probable). Safety assessments included clinical laboratory parameters (hematology, biochemistry, and urinalysis), vital signs (body temperature, blood pressure, heart rate, respiratory rate), physical examination, and 12-lead electrocardiographs (ECGs).

Results

Participant disposition

The study was conducted between May 2018 and May 2019. A total of 24 healthy participants in three consecutive cohorts of eight participants were enrolled and inoculated with *P. falciparum*-infected erythrocytes on day 0 (Fig. 1). Following inoculation, participants were randomized within each cohort (non-blinded) to a dose group. Dose combinations tested in cohort 1 were pre-defined in the study protocol while dose combinations tested in cohorts 2 and 3 were decided following preliminary PK/PD data assessment from the previous cohort. All participants received the allocated single oral dose of artefenomel and piperaquine on day 8. Two groups received the same dose (cohort 1D and cohort

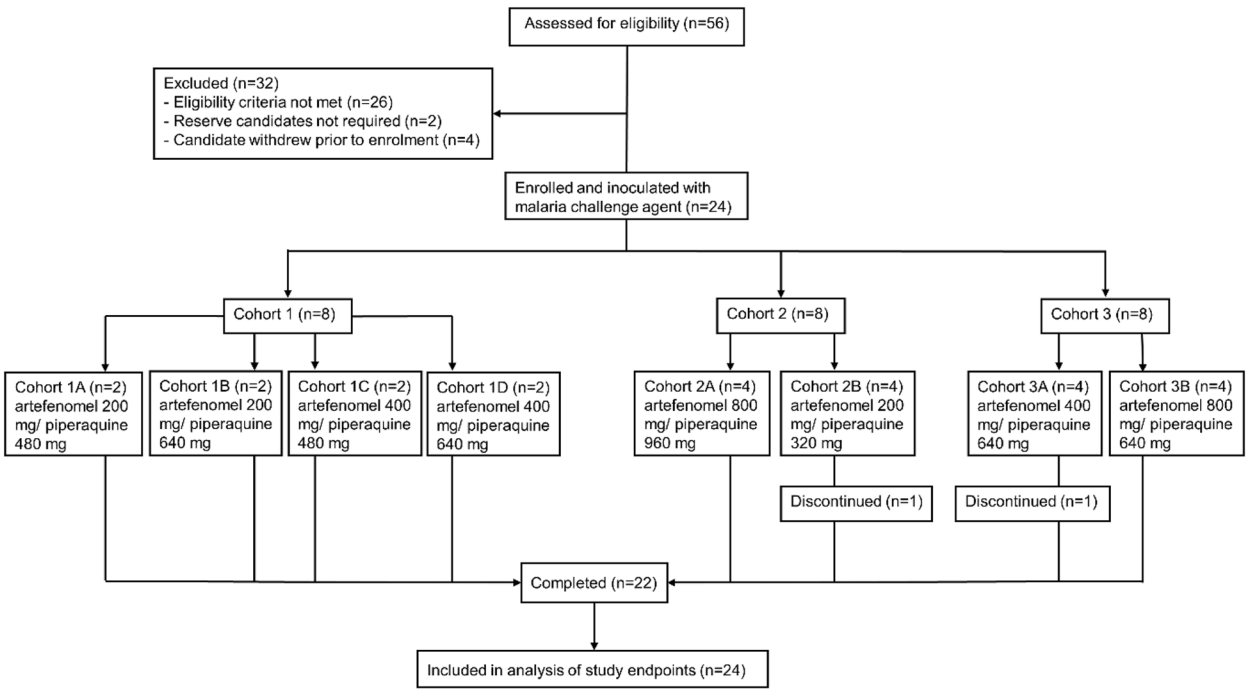

Fig. 1 Trial profile. Enrolled participants were randomized within each cohort to a dose group on the day of dosing with artefenomel + piperaquine combination treatment (8 days following challenge with blood-stage *P. falciparum*). Two participants discontinued voluntarily prior to the end of the study; available data from both participants were included in the analysis of study endpoints

Table 1 Demographic profile of participants

Parameter		Artefenomel 200 mg + piperaquine 480 mg (*N* = 2)	Artefenomel 200 mg + piperaquine 640 mg (*N* = 2)	Artefenomel 400 mg + piperaquine 480 mg (*N* = 2)	Artefenomel 400 mg + piperaquine 640 mg (*N* = 6)	Artefenomel 800 mg + piperaquine 960 mg (*N* = 4)	Artefenomel 200 mg + piperaquine 320 mg (*N* = 4)	Artefenomel 800 mg + piperaquine 640 mg (*N* = 4)	Total (*N* = 24)
Age (years)	Mean (SD)	36.5 (7.8)	23.0 (1.4)	40.5 (17.7)	23.5 (4.1)	21.3 (2.2)	23.8 (3.6)	20.0 (1.4)	25.0 (7.9)
Sex (*n* (%))	Male	2 (100)	2 (100)	1 (50.0)	6 (100)	3 (75.0)	2 (50.0)	2 (50.0)	18 (75.0)
	Female	0	0	1 (50.0)	0	1 (25.0)	2 (50.0)	2 (50.0)	6 (25.0)
Race (*n* (%))	White	1 (50.0)	0	2 (100)	5 (83.3)	3 (75.0)	4 (100)	4 (100)	19 (79.2)
	Asian	1 (50.0)	2 (100)	0	1 (16.7)	0	0	0	4 (16.7)
	Multiple races	0	0	0	0	1 (25.0)	0	0	1 (4.2)
BMI (kg/m^2)	Mean (SD)	25.0 (3.4)	22.5 (2.4)	22.1 (2.2)	23.2 (2.4)	22.0 (2.7)	25.1 (1.1)	23.1 (3.1)	23.3 (2.4)
Height (cm)	Mean (SD)	179.5 (16.3)	172.5 (0.7)	174.0 (4.2)	179.5 (8.7)	178.5 (7.9)	175.3 (6.3)	174.0 (5.5)	176.7 (7.3)
Weight (kg)	Mean (SD)	81.9 (25.4)	66.9 (6.7)	67.0 (9.9)	74.5 (6.5)	70.0 (9.6)	77.0 (4.6)	70.1 (10.9)	72.8 (9.7)

BMI Body mass index, *SD* Standard deviation

3A; 400 mg artefenomel and 640 mg piperaquine) and were combined for data analysis. Most participants were male (18/24, 75.0%) and self-selected their race as white (19/24, 79.2%); the mean age of participants was 25.0 years (Table 1).

Parasitemia and drug exposure

The progression of parasitemia following inoculation with blood-stage *P. falciparum* was consistent between participants, with an expected increase in parasitemia up to artefenomel + piperaquine administration on day 8 (Fig. 2). Dose-related increases in artefenomel and piperaquine exposure were observed across the dose range tested (Fig. 3A, B). Maximum plasma concentrations occurred 2–3 h after dosing for artefenomel and 3–5 h after dosing for piperaquine (Additional file 2: Table S4). An initial rapid fall in parasitemia occurred in all participants following dosing (Fig. 2), with a trend of increased rate of parasite clearance with increased dose of the combination (Table 2 and Additional file 2: Table S5). Parasite regrowth occurred after dosing with artefenomel 200 mg + piperaquine 320 mg (4/4 participants; 5 days post-dose), artefenomel 200 mg + piperaquine 480 mg (2/2 participants; 4 and 14 days post-dose), and artefenomel 400 mg + piperaquine 480 mg (1/2 participants; 14 days post-dose). Parasite regrowth was not observed in any of the other dose groups up to 35 ± 2 days post-dosing when definitive antimalarial treatment with artemether-lumefantrine was initiated. One participant in the artefenomel 200 mg + piperaquine 640 mg group exhibited low-level male and female gametocytemia after dosing (gametocytemia was distinguished from asexual parasite regrowth using qRT-PCR targeting gametocyte specific transcripts [data not shown]). One participant in the artefenomel 400 mg + piperaquine 640 mg group voluntarily withdrew from the study 7 days post-dosing requiring early artemether-lumefantrine treatment. One participant in the artefenomel 200 mg + piperaquine 320 mg group voluntarily withdrew from the study 3 days after artemether-lumefantrine had been administered for parasite regrowth. Available data from both participants who withdrew prior to the end of the study were included in the PK/PD analysis. All participants were confirmed to be aparasitemic by the end of the study.

Pharmacokinetic/pharmacodynamic modeling

The VIS PK/PD model was built using data from two prior VIS, in which artefenomel [8] and piperaquine [14] were administered as monotherapy, as well as data obtained in the current study. Population PK modeling using VIS data indicated that the PK profiles of artefenomel and piperaquine were appropriately described by a two-compartmental distribution model with linear elimination and first-order absorption (parameter estimates presented in Additional file 2: Table S6; visual predictive checks presented in Additional file 3: Fig. S2–S5). Models including inhibition of piperaquine clearance by artefenomel, inhibition of artefenomel clearance by piperaquine, or inhibition of clearance of both drugs by the other were tested. As none of the inhibition terms improved the model fit as measured by BIC, no PK drug-drug interaction was included in the model (difference in BIC between each of the three PK interaction models and the selected no PK interaction model was 7.58, 14.29, and 13.46, respectively). The PD of artefenomel and piperaquine were described by sigmoidal (E_{max}) concentration-killing relationships, with parameters estimated based on the respective monotherapy VIS data (Additional file 2: Table S7). The concentrations with half-maximal killing effect (EC_{50}) were estimated to be 2.89 ng/mL for artefenomel and 6.26 ng/mL for piperaquine. The maximum killing rate (E_{max}) for each drug was comparable (0.194/h for artefenomel and 0.262/h for piperaquine). A GPDI model, quantifying the mutual modulation of EC_{50} and E_{max}, described the parasitemia time courses for combination treatment (Additional file 3: Fig S6). In this model, the estimated interaction parameters were negative (Additional file 2: Table S7), suggesting synergy of potency (both drugs mutually decreased the EC_{50} of the other drug by 11.7%), but antagonism of efficacy (both drugs mutually decreased the E_{max} of the other drug by 40.6%), for the two compounds in combination (difference in BIC between no PD interaction model and the selected interaction model was −167.46). The interaction parameters of the GPDI model were estimated with small uncertainty given the relative standard error was less than 30% (Additional file 2: Table S7).

(See figure on next page.)

Fig. 2 Individual participant parasitemia-time profiles. Participants were inoculated intravenously with *P. falciparum*-infected erythrocytes and were administered a single oral dose of artefenomel and piperaquine in combination after 8 days (indicated by the vertical dashed line). Parasitemia was monitored using qPCR targeting the gene encoding *P. falciparum*18S rRNA. Artemether-lumefantrine was administered in response to parasite regrowth (indicated by the vertical arrows) or 35 ± 3 days after artefenomel + piperaquine dosing if parasite regrowth was not observed (indicated by the vertical dotted line). For the purpose of graphing on a $\log_{10}$ logarithmic scale, time points at which parasitemia could not be detected were substituted with a value of 1 parasite/mL. [a]One participant received early artemether-lumefantrine treatment due to voluntary withdrawal from the study

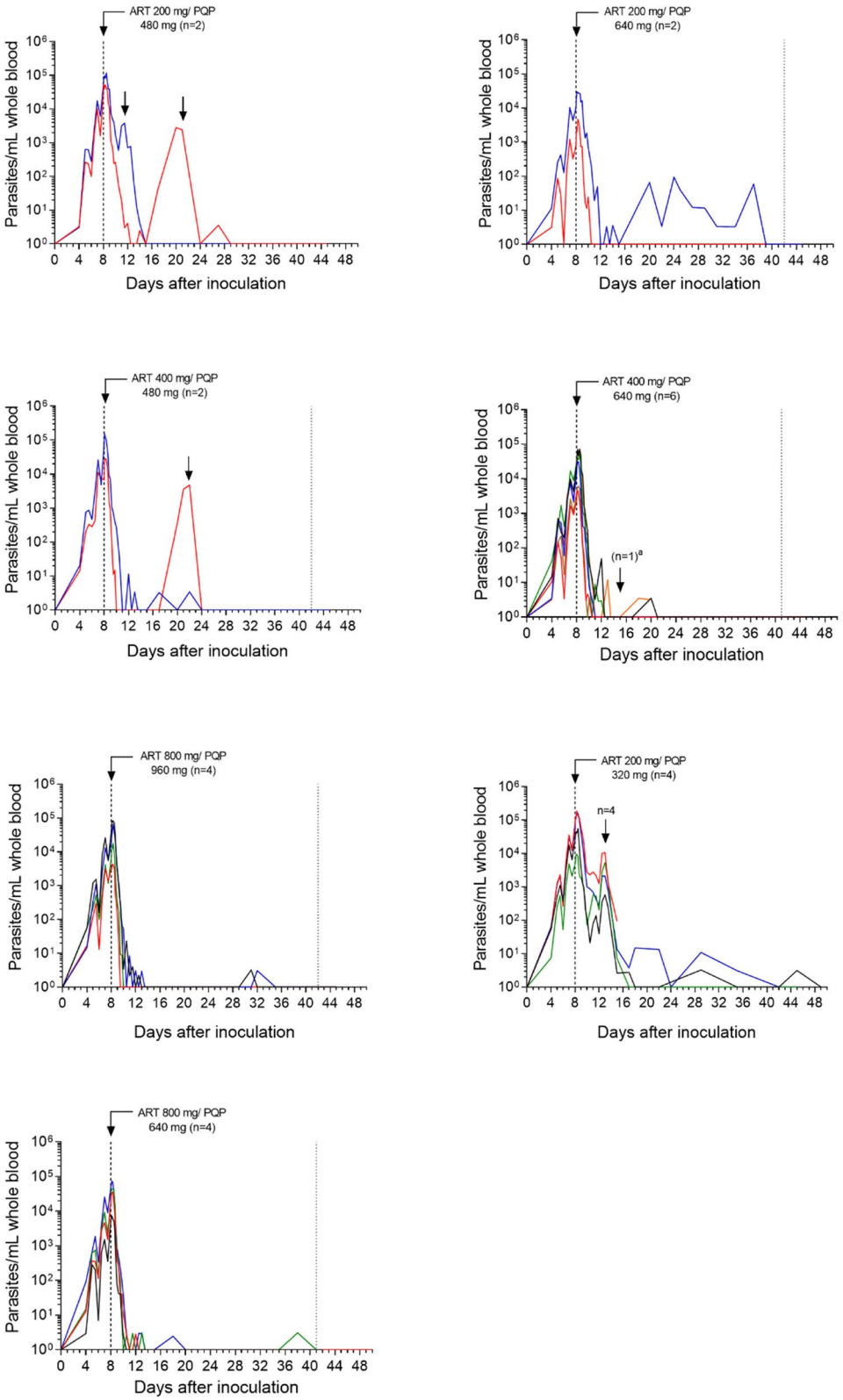

Fig. 2 (See legend on previous page.)

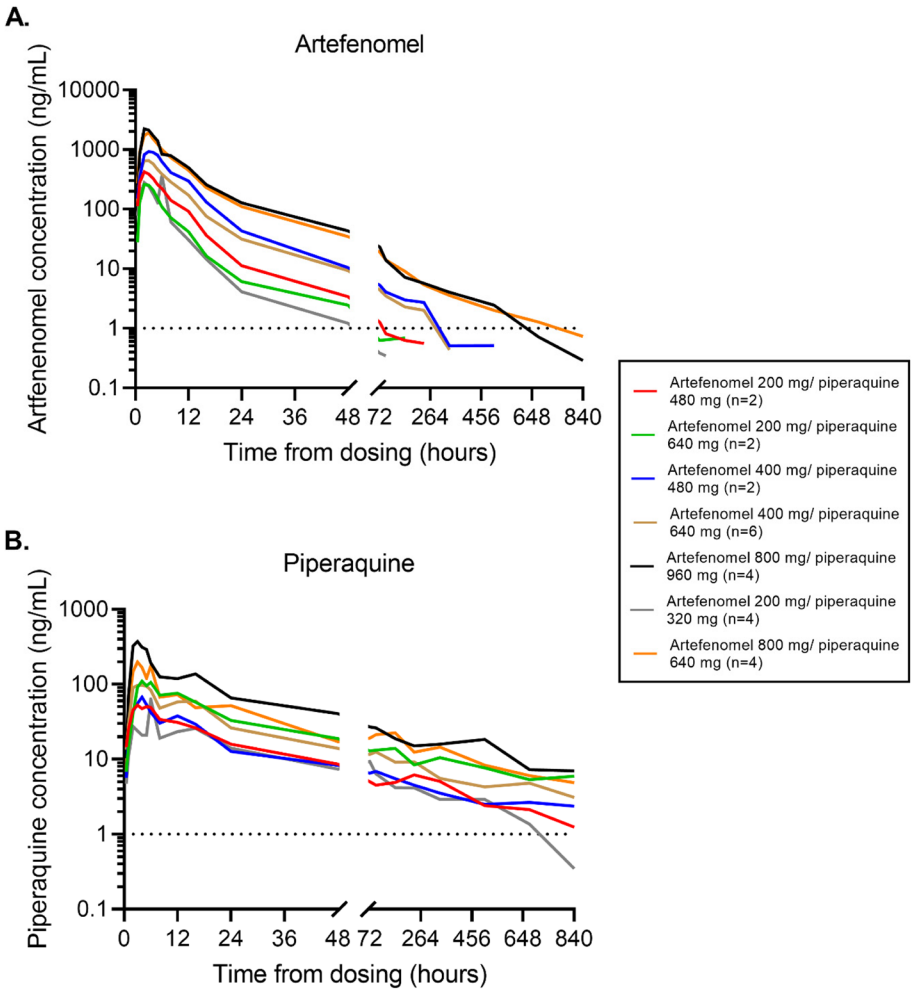

Fig. 3 Artefenomel and piperaquine plasma concentration–time profiles. Plots represent the arithmetic mean of the artefenomel (**A**) and piperaquine (**B**) plasma concentration of each dose group over the study. Plasma concentrations were measured using liquid chromatography with tandem mass spectrometry. The horizontal dotted lines indicate the lower limit of quantification (1 ng/mL)

Table 2 Parasite clearance kinetics and incidence of parasite regrowth

Treatment group	Log_{10} PRR_{48} (95% CI)	$PC_{t1/2}$ (hours) (95% CI)	Parasite regrowth (*n* (%))
Artefenomel 200 mg + piperaquine 480 mg (*N* = 2)	2.18 (2.00–2.36)	6.63 (6.13–7.22)	2 (100)
Artefenomel 200 mg + piperaquine 640 mg (*N* = 2)	2.34 (2.08–2.60)	6.18 (5.57–6.93)	0
Artefenomel 400 mg + piperaquine 480 mg (*N* = 2)	3.58 (3.29–3.86)	4.04 (3.74–4.39)	1 (50.0)
Artefenomel 800 mg + piperaquine 960 mg (*N* = 4)	3.98 (3.74–4.23)	3.63 (3.41–3.87)	0
Artefenomel 200 mg + piperaquine 320 mg (*N* = 4)	1.89 (1.75–2.04)	7.63 (7.10–8.25)	4 (100)
Artefenomel 800 mg + piperaquine 640 mg (*N* = 4)	3.56 (3.36–3.76)	4.06 (3.84–4.30)	0
Artefenomel 400 mg + piperaquine 640 mg (*N* = 6)	3.70 (3.52–3.88)	3.90 (3.72–4.10)	0

PRR_{48} parasite reduction ratio over a 48 h period, *$PCt_{1/2}$* parasite clearance half-life

Simulation of clinical success rates in patients

The probability of adequate parasitological response at day 28 (APR_{28}) was simulated for patients with acute uncomplicated *P. falciparum* malaria enrolled in the phase 2b trial of artefenomel + piperaquine combination therapy using a PK/PD model incorporating the PD interaction parameters estimated from the current VIS and individual drug PD parameters estimated from VIS monotherapy data. These PD parameters were combined with either the PK data derived from the VIS or in a separate simulation, with actual PK data derived from the patients in the phase 2b trial. Simulations were compared with the observed clinical success rates in the phase 2b trial to determine the predictive performance of the model. Simulations incorporating the VIS PK data highly overestimated the observed clinical success rates, with APR_{28} rates of 100% for each of the three dose combination groups (Table 3). This result was consistent with the fact that the plasma levels achieved for both artefenomel and piperaquine were higher in VIS participants than those achieved in patients in the phase 2b trial for equivalent dose combinations (Additional file 3: Fig. S7–S8). Simulations incorporating observed PK data from patients in the Phase 2b trial predicted the probability of APR_{28} across the three treatment groups with a high degree of accuracy (Table 3). The observed APR_{28} rate in the phase 2b trial was 67.0% for artefenomel 800 mg + piperaquine 640 mg, 65.5% for artefenomel 800 mg + piperaquine 960 mg, and 75.4% for artefenomel 800 mg + piperaquine 1440 mg. The simulated APR_{28} rates were 69.4%, 63.9%, and 74.8%, respectively. Combined effects (i.e., parasite killing rates) of the simulated median concentrations of artefenomel and piperaquine (based on the individual patients' PK Bayes estimates from phase 2b study) and PD and interaction parameters estimated from the VIS data are illustrated in Additional file 3: Fig. S9.

Safety and tolerability

A total of 101 AEs were reported during the study, with 21/24 participants (87.5%) experiencing at least one AE (Table 4 and Additional file 2: Table S8). All AEs were mild or moderate in severity; none were graded as severe or met the criteria for a SAE. There was no obvious relationship between the dose of artefenomel + piperaquine and the incidence of AEs. The majority of AEs were considered related to malaria (67/101 AEs). There were 7 AEs considered related to artefenomel + piperaquine dosing, experienced by 5 participants over two dose groups (artefenomel 800 mg + piperaquine 960 mg and artefenomel 400 mg + piperaquine 480 mg). These AEs were 5 events of mild nausea and one event each of mild headache and mild QT prolongation on ECG. The case of QT prolongation occurred in a participant dosed with 800 mg artefenomel and 960 mg piperaquine; the participant had a pre-dose QT interval (heart rate corrected using Fridericia's formula; QTcF) of 422 ms which increased to 450 ms and 460 ms at 4 and 6 h post-dosing respectively, before normalizing at 8 h post-dosing (425 ms).

Decreased white blood cell counts were observed in several participants; these were mild to moderate in severity and typically occurred within 4 days following dosing. Decreases in lymphocyte counts occurred in four participants (lowest nadir of the four: 0.55×10^9/L; normal range $1.0–4.0 \times 10^9$/L), decreases in neutrophil counts in two participants (lowest nadir of the two: 1.22×10^9/L; normal range $1.5–8.0 \times 10^9$/L), with a composite decrease in leukocyte count in one participant (2.9×10^9/L; normal range $3.5–12.0 \times 10^9$/L). Additionally, a mild decrease in hemoglobin from baseline (day 0, malaria challenge) was recorded for 4 participants (fractional fall 15–18% at nadir). These clinical laboratory abnormalities were all transient and considered to be probably or possibly related to malaria, and unrelated to combination dosing. No clinically significant elevations in liver function enzymes were recorded.

Discussion

The purpose of this study was to retrospectively evaluate whether characterizing the pharmacological interaction of artefenomel and piperaquine in a small number of healthy, malaria-naive adult volunteers using the *P. falciparum* IBSM model could have predicted the results

Table 3 Probability of APR_{28} based on patient data from a phase 2b combination trial compared to PK/PD model simulations

	Clinical trial	PK/PD model (VIS PK)	PK/PD model (Patient PK)
Artefenomel 800 mg + piperaquine 640 mg	67.0 (57.2–75.8)	100 (98.1–100)*	69.4 (65.4–74.1)
Artefenomel 800 mg + piperaquine 960 mg	65.5 (56.3–74.0)	100 (99.2–100)*	63.9 (60.5–68.9)
Artefenomel 800 mg + piperaquine 1440 mg	75.4 (66.6–82.9)	100 (99.2–100)*	74.8 (74.8–80.7)

Estimated probability is denoted in % with 95% confidence interval in parentheses. For clinical trial data, the estimated probability of event occurrence and the 95% binomial confidence intervals are shown. For the PK/PD simulations, the mean probability of event occurrence 0.025th and 0.975th quantile from 250 simulated trials are shown. The results of each PK/PD model simulation were compared to clinical trial data using a two-proportions *z*-test with the Benjamini–Hochberg procedure (*indicates *p*-value < 0.05). *APR_{28}*, adequate parasitological response on day 28

Table 4 Summary of adverse events

Adverse event type[a]	Artefenomel 200 mg + piperaquine 480 mg (*N* = 2)	Artefenomel 200 mg + piperaquine 640 mg (*N* = 2)	Artefenomel 400 mg + piperaquine 480 mg (*N* = 2)	Artefenomel 400 mg + piperaquine 640 mg (*N* = 6)	Artefenomel 800 mg + piperaquine 960 mg (*N* = 4)	Artefenomel 200 mg + piperaquine 320 mg (*N* = 4)	Artefenomel 800 mg + piperaquine 640 mg (*N* = 4)	Total (*N* = 24)
Participants with at least one AE (%); number of AEs								
Any AE	2 (100); 10	2 (100); 3	2 (100); 14	4 (66.7); 16	4 (100); 22	3 (75.0); 19	4 (100); 17	21 (87.5); 101
Grade 2 AE[b]	0	0	1 (50.0); 4	2 (33.3); 2	2 (50.0); 2	3 (75.0); 7	2 (50.0); 3	10 (41.7); 18
AE related to malaria	2 (100); 9	0	2 (100); 13	4 (66.7); 11	4 (100); 9	3 (75.0); 14	4 (100); 11	19 (79.2) 67
AE related to ART/PQP[c]	0	0	2 (100); 3	0	3 (75.0); 4	0	0	5 (20.8); 7
Nausea	0	0	2 (100); 2	0	3 (75.0); 3	0	0	5 (20.8); 5
QT prolongation on ECG	0	0	0	0	1 (25.0); 1	0	0	1 (4.2); 1
Headache	0	0	1 (50.0); 1	0	0	0	0	1 (4.2); 1

[a] No adverse events met the criteria for a serious adverse event (SAE) or resulted in study discontinuation

[b] The severity of adverse events was graded in accordance with the Common Terminology Criteria for Adverse Events v4.03 (grade 1 = mild, grade 2 = moderate, grade 3 = severe, grade 4 = potentially life-threatening, grade 5 = death related to adverse event); no grade 3, grade 4 or grade 5 adverse events occurred in this study

[c] All adverse events considered related to artefenomel + piperaquine combination treatment were grade 1 (mild) in severity. *AE*, adverse event; *ECG*, electrocardiograph

of a large phase 2b combination study [13]. The phase 2b study was conducted in 7 countries over two continents (Africa and Asia). It enrolled a total of 448 patients (the majority of whom were children under the age of 5 years), with patient recruitment and follow-up occurring over a 12-month period. Dose selection was primarily designed to achieve the maximum exposure that would be well tolerated, with minimization of a potential prolongation of the QT interval by high plasma levels of piperaquine a key consideration [13, 28]. The negative outcome of the trial was a significant disappointment given the implications with respect to the development of an alternative treatment to ACTs and overall progress towards the goal of malaria eradication. Here, we demonstrate that a PK/PD model of artefenomel + piperaquine developed from a VIS predicted the efficacy of the combination in the phase 2b study with a high degree of accuracy, if PK data from the target population were utilized for the simulations.

In this VIS, the pharmacological interaction between artefenomel and piperaquine was characterized using an adaptive study design to test multiple dose combinations, with dose selection informed by preliminary analyses of preceding dose groups. PK/PD modeling activities were enabled by having the PK and PD data on each compound administered alone, obtained from prior *P. falciparum* IBSM VIS [8, 14]. Consistent with previous in vitro drug-drug interaction assessment and the population PK model built from the phase 2b combination trial [13], no PK drug-drug interaction between artefenomel and piperaquine was identified using population PK modeling in the current study. A PK/PD interaction model (GPDI model) described the parasitemia time courses following combination treatment and indicated synergistic activity between the two drugs with respect to potency, but antagonistic activity with respect to maximum parasite killing rate. This interaction model was combined with PD models of artefenomel and piperaquine generated from previous monotherapy VIS data for simulations to predict clinical success rates at day 28 for the completed phase 2b combination trial.

Importantly, we found that the ability of the VIS PK/PD model to accurately predict the results of the phase 2b trial was highly dependent on the source of the PK data used in the simulations. When the PK model built from VIS data was extrapolated to the patient population, the simulations greatly overestimated clinical success compared to the observed results in the phase 2b trial, with APR_{28} rates of 100% for each of the three dose combination groups in the trial. However, when the simulations utilized actual PK data from patients in the phase 2b trial, the observed clinical success rates were accurately predicted. These findings indicated that while the PK/PD relationship of the combination characterized in the VIS was directly translatable to the patient population, the PK profile of the combination was significantly different between VIS participants and patients.

The finding that utilizing PK data from the VIS for simulations in patients resulted in an overestimation of the clinical success of the combination was due to the fact that both artefenomel and piperaquine plasma exposures were higher in VIS participants compared with the patient population. This is not surprising, given the expected differences in PK between the healthy adult participants (predominantly Caucasian) in the VIS and the African and Asian patients with uncomplicated *P. falciparum* malaria (predominantly children ≤ 5 years of age). Although body weight was accounted for when using the VIS PK model for simulations in patients, other factors (altered drug absorption, vomiting, and developmental, genetic, and physiological factors influencing biodistribution and clearance) are likely responsible for the differences in observed PK profiles. Indeed, the authors of the phase 2b trial noted that insufficient compliance data on drug consumption were collected, with anecdotal reports that young children were unable to ingest the full dose, as well as a higher than expected rate of vomiting.

In addition to differences in drug exposure, a number of other factors could be expected to impact the translatability of findings from malaria VIS to studies in malaria-endemic settings, including immunity, concomitant therapies, or parasite drug resistance. Regarding the latter, the current VIS utilized the *P. falciparum* 3D7 strain known to be fully sensitive to both artefenomel and piperaquine, as well as all commonly used antimalarials. In the phase 2b study, the efficacy of artefenomel-piperaquine was lower in the Vietnamese population relative to the African population [13], and the authors hypothesized that this may have been due to the high frequency of Kelch13 mutant parasites identified in the Vietnamese population, and the prevalence of piperaquine resistant parasites in this geographic region (the frequency of piperaquine resistant parasites in the study was not directly determined). Evidence for cross-over between artemisinin resistance (conferred by Kelch13 mutations) and artefenomel resistance was demonstrated, with Kelch13 mutant parasites cleared at a slower rate than their wild type counterparts following combination dosing. However, Kelch13 mutation was not found to be a significant covariate for the overall efficacy of the combination (clinical success at day 28), with small sample size being a potential caveat in this result. In the current study, drug resistance or other factors such as immunity were not accounted for in the PK/PD model. Despite this, the simulations based on the VIS PK/PD model closely matched the observed efficacy results in the phase 2b

study (when taking into consideration the actual drug exposure data) suggesting that for this study these factors did not impact translatability. However, we do not discount the possibility that the performance of a VIS PK/PD model may be less robust in other populations or with other drug combinations.

Conclusions

This study has demonstrated that characterizing the pharmacological interaction of artefenomel + piperaquine in a VIS could have predicted the outcome of the selected dose combinations in the phase 2b study, provided that PK data from the target patient population were available. The value of assessing the pharmacological interaction between antimalarial combinations during in vitro parasite culture, and in the humanized mouse model of malaria, to inform clinical decision-making around drug and dose selection has recently been established [29, 30]. However, the translatability of these models is likely to be limited by the complex PK/PD interactions that may occur in humans. Therefore, performing a VIS to characterize the PK/PD relationship of a promising new antimalarial combination, in conjunction with a PK bridging study in the target patient population (including both young children and adults), may be a worthy strategy to optimize resources and maximize successful outcomes of subsequent phase 2 and 3 trials.

Abbreviations

ACT	Artemisinin-based combination therapy
$ACPR_{28}$	Adequate clinical and parasitological response at day 28
AEs	Adverse events
APR_{28}	Adequate parasitological response at day 28
EC_{50}	Concentration with half-maximal killing effect
ECG	Electrocardiograph
E_{max}	Maximum killing rate
GPDI	General pharmacodynamic interaction
IBSM	Induced blood-stage malaria
LLOQ	Lower limit of quantification
$PC_{t1/2}$	Parasite clearance half-life
PD	Pharmacodynamic
PK	Pharmacokinetic
PRR_{48}	Parasite reduction ratio over 48 h
qPCR	Quantitative polymerase chain reaction
SAE	Serious adverse event
VIS	Volunteer infection study

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-024-03787-0.

Additional file 1: Text S1. Participant eligibility criteria. Text S2. Definition of adequate parasitological response on day 28 .

Additional file 2: Table S1. Schedule of study activities and procedures. Table S2. Volunteer infection study data used for pharmacokinetic/pharmacodynamic modelling of artefenomel-piperaquine combination. Table S3. Phase 2b study data used in simulations to predict APR28 in patients. Table S4. Plasma artefenomel and piperaquine non-compartmental pharmacokinetic parameters. Table S5. Individual participant parasite clearance parameters. Table S6. Parameter estimates for the final pharmacokinetic combination model of artefenomel and piperaquine from monotherapy and combination therapy volunteer infection study data. Table S7. Parameter estimates of the pharmacokinetic/pharmacodynamic model for artefenomel and piperaquine in monotherapy and in combination. Table S8. Adverse events by system organ class and preferred term.

Additional file 3: Figure S1. Graphical overview of the PK/PD modelling approach and simulations performed. Figure S2. Visual predictive checks for artefenomel concentration–time profiles from observed results when administered as monotherapy and simulations using the VIS PK model. Figure S3. Visual predictive checks for piperaquine concentration–time profiles from observed results when administered as monotherapy and simulations using the VIS PK model. Figure S4. Visual predictive checks for artefenomel concentration–time profiles from observed results when administered in combination with piperaquine and simulations using the VIS PK model. Figure S5. Visual predictive checks for piperaquine concentration–time profiles from observed results when administered in combination with artefenomel and simulations using the VIS PK model. Figure S6. Individual fits for participants in the artefenomel + piperaquine combination volunteer infection study. Figure S7. Artefenomel plasma concentration–time profiles by body weight of patients in the phase 2b trial compared to the PK model built from VIS data. Figure S8. Piperaquine plasma concentration–time profiles by body weight of patients in the phase 2b trial compared with the PK model built from VIS data. Figure S9. Parasite killing rate as a function of artefenomel and piperaquine concentration.

Additional file 4.

Additional file 5.

Additional file 6.

Additional file 7,

Additional file 8.

Additional file 9.

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Authors' contributions

ANA, DK, and AK performed the pharmacokinetic/pharmacodynamic modeling. AO, SW, and BEB performed the clinical components of the study. SL, LW, and LM performed the analysis of parasite clearance. SC was responsible for medical oversight. MEG, JSM, and JJM were responsible for study conceptualization and study design. RW performed project management activities. AJP led manuscript development. All authors read and approved the final manuscript.

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Data availability

All data analyzed during the current study are either presented in the main text or included as additional files. Additional files 4–9 include the raw data and code used for PK/PD modeling and simulations.

Declarations

Ethics approval and consent to participate

The study was approved by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee (reference number: P2370). All participants gave written informed consent before enrolment.

Consent for publication

Not applicable.

Competing interests

JJM, MEG, and SC are currently employed by Medicines for Malaria Venture (MMV) which funded the study. BEB and JSM received funding from MMV to perform the study. All other authors declare no competing interests.

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