MALARIA

The pyrrolidinamide antimalarial drug MMV367 rapidly clears blood-stage *Plasmodium falciparum* in healthy adults with experimental malaria

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Fast-acting antimalarial drugs are needed to address the emergence of artemisinin resistance in the protozoan parasite *Plasmodium falciparum,* the major cause of severe malaria worldwide. The pyrrolidinamide antimalarial drug MMV367 (GSK3772701) potentially interferes with parasite metabolism mediated by *P. falciparum* acyl-CoA synthetases 10 and 11. In this volunteer infection study, the antimalarial activity of MMV367 was tested against blood-stage *P. falciparum* in 12 healthy malaria-naïve adults. The study participants were inoculated with *P. falciparum* 3D7–infected erythrocytes on day 0 and received a single oral dose of MMV367 at 3 mg ($n = 3$), 5 mg ($n = 2$), 10 mg ($n = 1$), 20 mg ($n = 3$), 90 mg ($n = 2$), or 1500 mg ($n = 1$) on day 8. All participants received artemether-lumefantrine on or before day 24. Maximum MMV367 plasma concentrations occurred 2 to 4 hours postdose, and the elimination half-life was 12.9 to 18 hours. For doses of ≥20 mg, the parasite clearance half-life was 2.2 to 4.3 hours with viable parasites declining more rapidly (elimination half-life of 1.1 to 2.7 hours). A clearance pharmacokinetic/pharmacodynamic model described the relationship between MMV367 concentrations and parasite killing and subsequent clearance of nonviable parasites. Model-derived efficacy parameters included a minimum inhibitory concentration of 6.3 ng/ml (95% CI, 5.2 to 7.8) and a minimum parasiticidal concentration with 90% of maximum effect of 54 ng/ml (95% CI, 43 to 68). No reduction in in vitro susceptibility to MMV367 or genetic determinants of resistance were observed in drug-exposed parasite lines. Most adverse events were attributed to malaria challenge, and none were serious. Higher MMV367 doses were not associated with an increased incidence or severity of adverse events. These findings support further clinical development of MMV367.

INTRODUCTION

Malaria continues to be responsible for a large global health burden, with an estimated 282 million cases and 610,000 deaths in 2024 (*1*). The emergence of artemisinin-resistant *Plasmodium falciparum* in Southeast Asia (*2*), and more recently in East Africa (*3*–*5*), threatens the utility of the current first-line artemisinin-based combination therapies (ACTs). Alternatives to artemisinins are urgently required to progress efforts toward reducing malaria morbidity and mortality.

Artemisinins are the fast-acting component of ACTs, capable of reducing the parasite load by a factor of 10,000 per 48-hour asexual blood-stage parasite cycle, thus quickly reducing the parasite density and limiting opportunities to select resistance to the partner drug (*6*, *7*), in addition to achieving a rapid clinical response. In areas with artemisinin-sensitive *P. falciparum*, the parasite clearance half-life after a 3-day treatment with the artemisinin-derivative artesunate (4 mg/kg per day) was found to range from 1.8 to 3 hours (*8*). A similar result was observed in healthy volunteers with experimental malaria infection, with a single oral dose (2 mg/kg) resulting in a parasite clearance half-life of 3.2 hours (*9*). New drugs replacing artemisinins should exhibit similar fast-acting and highly potent activity, with a different mode of antimalarial action. A longer elimination half-life would be desirable to maximize the time parasites are exposed to parasiticidal concentrations of more than one compound (*6*, *10*).

Pyrrolidinamides represent a new antimalarial drug class identified by high-throughput phenotypic screening of an extensive compound library (*11*). Mode-of-action studies suggest that pyrrolidinamides may interfere with parasite acyl–coenzyme A (CoA) synthetases 10 and 11, predicted to be essential for the synthesis of long-chain fatty acids in asexual blood-stage parasites (*12*). MMV367 (also known as GSK3772701 or MMV1582367) is a pyrrolidinamide that has undergone extensive preclinical development, demonstrating rapid activity against *P. falciparum*, no cross-resistance with current antimalarials, and an acceptable preclinical safety and tolerability profile; additionally, it has predicted pharmacokinetic (PK) properties compatible with use as an antimalarial drug. Together, these properties supported the progression of MMV367 to clinical development.

A first-in-human study to assess the safety, tolerability, and PK of oral MMV367 in healthy adults has been completed (*13*). This multipart study comprised a placebo-controlled single ascending dose, food effect, and 3-day dosing components. MMV367 was well tolerated

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across the dose range, including at the highest single dose tested of 1500 mg. There was no apparent relationship between dose and the incidence of treatment-emergent adverse events (AEs). Only two AEs were considered by the investigator to be related to MMV367 (abdominal pain and upper abdominal pain), in the same participant receiving a single 750-mg dose; both AEs resolved spontaneously. MMV367 exhibited an elimination half-life of 16.5 to 18.4 hours, with a 1.3-fold increase in exposure [area under the curve (AUC) up to the last measurable concentration] after a high-fat meal and 2.1-fold accumulation ratio (AUC within the dosing interval) after 400 mg once daily dosing over 3 days. The favorable safety and PK results obtained in this study supported the continued clinical development of MMV367.

Volunteer infection studies using the induced blood-stage malaria model have previously successfully characterized the PK and pharmacodynamic (PK/PD) relationship of several candidate antimalarial compounds and antimalarial drugs currently in use (*14*–*18*). The primary objective of the current study was to assess the antimalarial activity of MMV367 by determining the PK/PD relationship after administration to healthy adult participants using the *P. falciparum*–induced blood-stage malaria model.

RESULTS

Study overview and participants

The study was conducted from 6 July to 30 October 2023. Twelve participants were enrolled across two consecutive cohorts and intravenously inoculated with *P. falciparum* on day 0 (Fig. 1). Participants were healthy malaria-naïve males ($n = 6$) or females ($n = 6$) aged 18 to 49 years (Table 1). Participants were randomized to a MMV367 dose group on day 8 (3 mg, $n = 3$; 5 mg, $n = 2$; 10 mg, $n = 1$; 20 mg, $n = 3$; 90 mg, $n = 2$; or 1500 mg, $n = 1$). All 12 randomized participants received the allocated dose of MMV367, completed the study, and were included in the analysis of study end points.

MMV367 clears blood-stage *P. falciparum* in a dose-dependent manner

The progression of parasitemia after intravenous inoculation was relatively consistent between participants up to day 8 (Fig. 2, A to F), although one participant (participant 112, administered 5 mg of MMV367) exhibited a delayed onset of parasitemia and thus lower parasite density at the time of MMV367 administration (Fig. 2B). Dose-related increases in MMV367 exposure [maximum concentration (C_{max}) and area under the concentration-time curve from

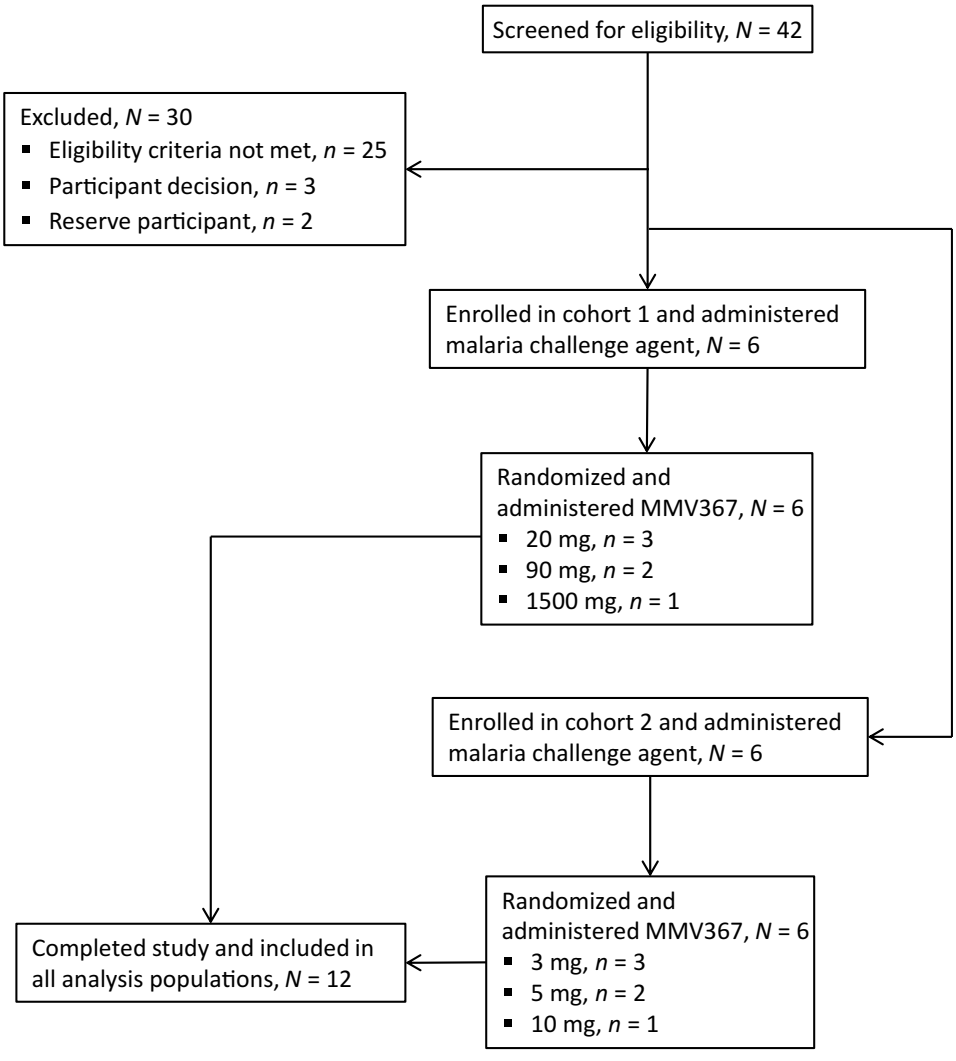

Fig. 1. CONSORT diagram for study. A total of 12 eligible participants were enrolled in one of two cohorts ($n = 6$ each) and challenged with blood-stage *P. falciparum*. Participants were randomized within each cohort to a MMV367 dose group on the day of dosing (8 days postchallenge). Participants were randomized to receive a single oral dose of MMV367 of 3 mg ($n = 3$), 5 mg ($n = 2$), 10 mg ($n = 1$), 20 mg ($n = 3$), 90 mg ($n = 2$), or 1500 mg ($n = 1$). All participants received the allocated dose of MMV367 and completed the study per protocol.

Table 1. Demographics and baseline characteristics of study participants.

		MMV367, 3 mg ($n = 3$)	MMV367, 5 mg ($n = 2$)	MMV367, 10 mg ($n = 1$)	MMV367, 20 mg ($n = 3$)	MMV367, 90 mg ($n = 2$)	MMV367, 1500 mg ($n = 1$)	Total ($n = 12$)
Sex [*n* (%)]	Female	2 (66.7%)	1 (50%)	0	2 (66.7%)	0	1 (100%)	6 (50%)
	Male	1 (33.3%)	1 (50%)	1 (100%)	1 (33.3%)	2 (100%)	0	6 (50%)
Race [*n* (%)]	White	3 (100%)	1 (50%)	1 (100%)	2 (66.7%)	2 (100%)	1 (100%)	10 (83.3%)
	Asian	0	1 (50%)	0	0	0	0	1 (8.3%)
	Multiple	0	0	0	1 (33.3%)	0	0	1 (8.3%)
Age (years)	Median (range)*	20 (18–22)	37 (24–49)	33	21 (19–31)	38 (29–47)	35	27 (18–49)
Body weight (kg)	Median (range)*	59 (53–69)	85 (82–88)	87	65 (57–85)	79 (77–81)	77	77 (53–88)
Body mass index (kg/m^2)	Median (range)*	20 (20–23)	27 (25–29)	26	21 (18–29)	25 (25–25)	28	25 (18–29)

*Range not presented for MMV367 10 mg– and 1500 mg–dose groups because $n = 1$.

time 0 to the last measured time point (AUC_{last})] were observed across the dose range (Fig. 2G and Table 2). Absorption was relatively rapid, with time to maximum plasma concentration (t_{max}) ranging from 2 to 4 hours, and an elimination half-life ($t_{1/2}$) ranging from 13 to 18 hours was observed.

In the first cohort ($n = 6$), a rapid reduction in parasitemia was observed for all participants administered 20, 90, or 1500 mg of MMV367 (Fig. 2, D to F). Parasite regrowth occurred for one of three participants dosed with 20 mg of MMV367, and definitive antimalarial treatment was initiated on day 22 (14 days after MMV367 dosing). This participant exhibited lower MMV367 exposure (C_{max} of 96.3 ng/ml, AUC_{last} of 1980 ng·hour/ml) compared with the other two participants administered 20 mg of MMV367 (C_{max} of 157 to 180 ng/ml, AUC_{last} of 4180 to 4200 ng·hour/ml). Parasite regrowth was not observed in any of the other five participants who received 20, 90, or 1500 mg of MMV367 through day 24, when compulsory definitive antimalarial treatment was initiated per protocol.

In the second cohort ($n = 6$), after the administration of a single oral dose of 3, 5, or 10 mg of MMV367, early definitive antimalarial treatment was administered between 1 and 4 days after dosing on the basis of the progression of parasitemia and clinical symptoms (five participants) or in response to a positive COVID-19 rapid antigen test result for one participant (Fig. 2, A to C). Gametocytemia was not detected for any participant after MMV367 dosing given that *pfs25* and *pfMGET* transcripts remained below the assay limit of detection at all time points assessed from day 12 onward.

MMV367 rapidly clears blood-stage parasitemia

The kinetics of parasite clearance were estimated using the slope of the optimal fit for the log-linear relationship of the parasitemia decay [as measured by quantitative polymerase chain reaction (qPCR)] for participants dosed with 10, 20, 90, or 1500 mg of MMV367 (Table 3). The derived parasite clearance half-life was 2.20 to 4.34 hours for doses of ≥20 mg, whereas slower clearance was observed for the single participant dosed with 10 mg of MMV367 (6.75 hours). A reliable estimate of parasite clearance kinetics associated with doses of 3 or 5 mg of MMV367 was not possible because poor parasite clearance resulted in a nonoptimal regression fit for most participants. However, data from these participants were important to optimally characterize the PK/PD relationship of MMV367.

Viable parasites decline at a faster rate than parasite clearance after MMV367 administration

The primary method for measuring parasite growth and clearance in this study was 18*S* qPCR. However, this method quantifies all parasites, including those nonviable or dead in circulation. Thus, we performed modeling of parasite growth observed ex vivo to determine the fraction of viable parasites present in blood samples. Viable parasites in peripheral blood were found to decline at a faster rate after MMV367 dosing compared with the rate that parasites were cleared (Table 2 and fig. S1). With MMV367 doses of ≥20 mg, viable parasite concentrations declined with an elimination half-life of 1.14 to 2.68 hours, with evidence of a dose effect [coefficient of determination (R^2) = 0.85, $P = 0.0085$, inverse-variance weighted regression, $n = 6$]. Similar to the observation for parasite clearance, a slower rate of decrease in viable parasite concentrations was observed for the participant dosed with 10 mg of MMV367 (6.79 hours).

In vivo exposure to MMV367 was not associated with detectable parasite drug resistance

Blood samples were collected from five participants immediately before definitive antimalarial treatment to investigate whether parasite resistance emerged after exposure to MMV367 in vivo. Samples were collected from participant 106 (20 mg of MMV367), who experienced parasite regrowth 14 days after MMV367 dosing, and from participants 110 (3 mg of MMV367), 109 (10 mg of MMV367), and 107 and 112 (5 mg of MMV367), who each received definitive antimalarial treatment between 2 and 4 days after dosing based on the progression of parasitemia and clinical symptoms (Fig. 2). Samples were not collected from participants 108 and 111 (3 mg of MMV367) because definitive antimalarial treatment was required 1 day after MMV367 dosing because of clinical symptoms and a positive COVID-19 test result, respectively. No evidence of reduced in vitro susceptibility to MMV367 was observed in any of the drug-exposed parasite lines compared to the control (table S1). Furthermore, the MMV367-exposed parasite lines did not display altered in vitro susceptibility to dihydroartemisinin,

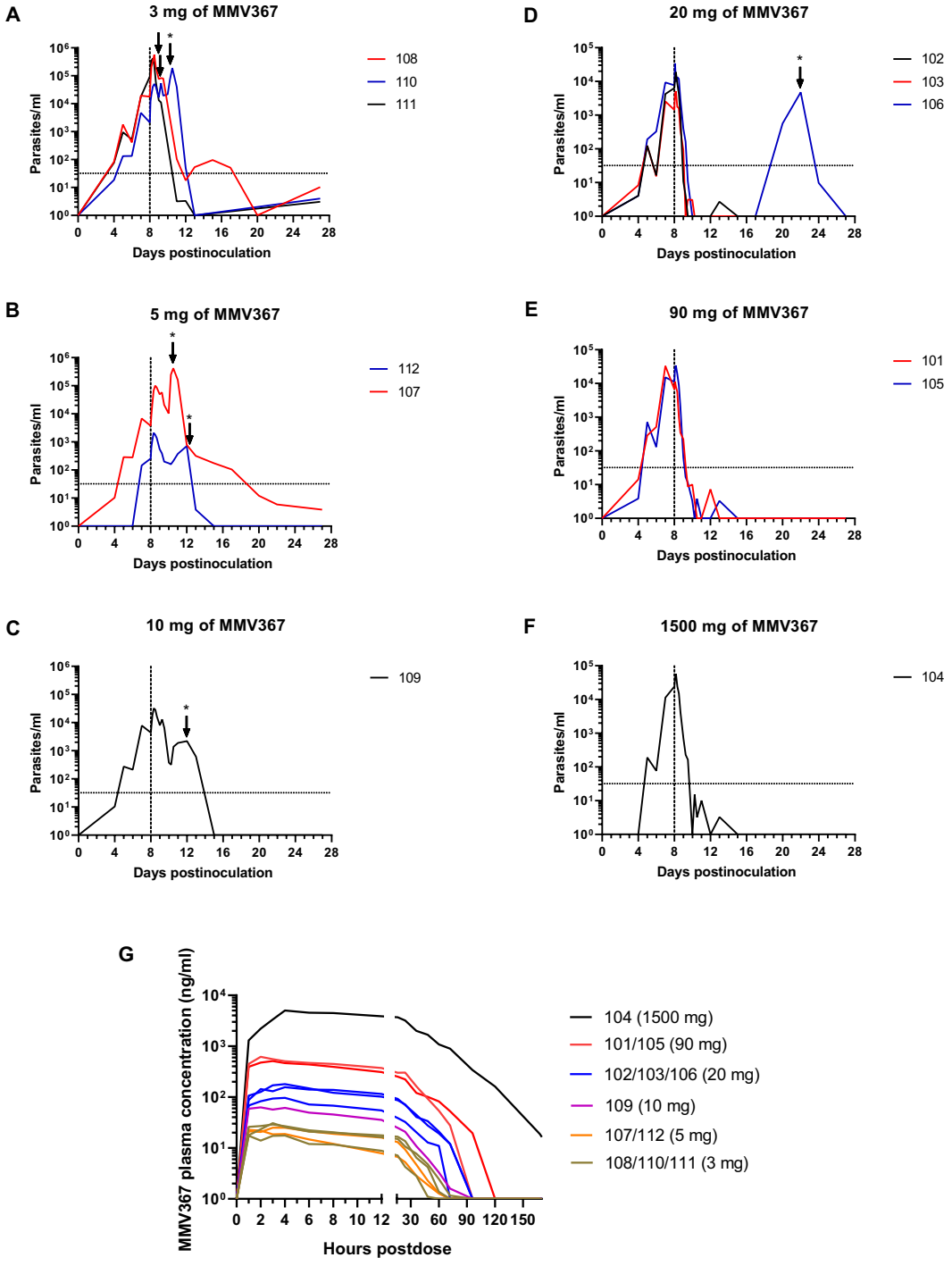

Fig. 2. MMV367 clears blood-stage *P. falciparum* in a dose-dependent manner in healthy volunteers. (**A** to **F**) Shown are individual participant parasitemia profiles and (**G**) individual participant MMV367 plasma concentration profiles. Participants were inoculated intravenously with *P. falciparum*–infected erythrocytes on day 0 and were administered a single oral dose of 3 mg ($n = 3$), 5 mg ($n = 2$), 10 mg ($n = 1$), 20 mg ($n = 3$), 90 mg ($n = 2$), or 1500 mg ($n = 1$) of MMV367 on day 8. Black arrows in (A) to (D) represent the early initiation of definitive antimalarial treatment with artemether-lumefantrine for seven participants. Participants 108 and 110 (3 mg of MMV367), 107 and 112 (5 mg of MMV367), and 109 (10 mg of MMV367) required early treatment because of the progression of parasitemia and clinical symptoms. Participant 106 (20 mg of MMV367) required early treatment because of parasite regrowth. Participant 111 (3 mg of MMV367) required early treatment because of a positive COVID-19 rapid antigen test. Participants 102 and 103 (20 mg of MMV367), 101 and 105 (90 mg of MMV367), and 104 (1500 mg of MMV367) all received definitive antimalarial treatment commencing on day 24 postchallenge per study protocol. Asterisks in (A) to (D) represent time points where a blood sample was collected to assess parasite drug resistance (before definitive antimalarial treatment). Parasitemia was measured using qPCR targeting the gene encoding *P. falciparum* 18*S* rRNA. The horizontal dotted lines in (A) to (F) indicate the lower limit of quantification of the assay (32 parasites/ml). Plasma MMV367 concentration was measured using liquid chromatography tandem mass spectrometry (lower limit of quantification was 10 ng/ml for doses of ≥20 mg or 1 ng/ml for doses of ≤10 mg).

Table 2. MMV367 plasma noncompartmental pharmacokinetic parameters associated with single-dose administration to healthy volunteers with *P. falciparum* parasitemia. Data are geometric means [coefficient of variation (%)] except t_{max}, which is median (range). CL/F, apparent total clearance; Vz/F, apparent volume of distribution.

	MMV367, 3 mg ($n = 3$)	MMV367, 5 mg ($n = 2$)	MMV367, 10 mg ($n = 1$)*	MMV367, 20 mg ($n = 3$)	MMV367, 90 mg ($n = 2$)	MMV367, 1500 mg ($n = 1$)*
C_{max} (ng/ml)	25.0 (31.4)	23.8 (7.1)	62.9	140 (33.8)	562 (13.5)	5,050
t_{max} (h)	3.0 (3.0–4.0)	2.0 (1.0–3.0)	2.0	4.0 (4.0–4.0)	2.5 (2.0–3.0)	4.0
AUC_{0-last} (h × ng/ml)	509 (49.7)	429 (31.1)	1,260	3264 (45.4)	14,443 (4.4)	194,000
$t_{1/2}$ (h)	12.9 (11.8)	16.5 (32.1)	13.1	18.0 (10.1)	15.6 (14.6)	17.4
CL/F (ml/h)	5,633 (47.8)	10,805 (26.6)	7,750	5,575 (40.6)	6,017 (4.7)	7,690
Vz/F (ml)	104,853 (46.9)	257,249 (62.9)	147,000	145,346 (47.2)	135,241 (19.5)	193,000

*Summary statistics were not calculated for the MMV367 10-mg and 1500-mg groups because $n = 1$.

Table 3. Parasite clearance half-life and viable parasite elimination half-life after single-dose MMV367 administration to healthy volunteers with *P. falciparum* parasitemia. Results for the MMV367 3 mg– and 5 mg–dose groups are not presented because analyses revealed nonsignificant parasite clearance and viable parasite elimination for several participants.

MMV367 dose	Participant ID	Parasite clearance half-life, hours (95% CI)	Viable parasite elimination half-life, hours (95% CI)
1500 mg	104	3.11 (2.72–3.64)	1.14 (0.87–1.68)
90 mg	101	4.34 (3.80–5.06)	1.87 (1.32–3.23)
90 mg	105	3.33 (2.86–3.99)	1.71 (1.22–2.83)
20 mg	102	2.20 (1.88–2.65)	2.01 (1.46–3.20)
20 mg	103	3.72 (3.09–4.68)	2.68 (1.75–5.68)
20 mg	106	3.07 (2.70–3.55)	2.06 (1.55–3.04)
10 mg	109	6.75 (5.28–9.35)	6.79 (5.26–9.59)

monodesethyl-amodiaquine, or pyronaridine relative to the drug-sensitive 3D7-A parental *P. falciparum* strain (table S2).

Whole-genome sequencing was performed to search for genetic determinants associated with resistance to MMV367. High-quality genomic coverage was obtained, with a mean read depth ranging from 27.7 to 40.0 per nucleotide across all samples (table S3). No new high-confidence single-nucleotide polymorphisms (SNPs) were identified in any of the drug-exposed parasite genomes relative to the parental strain. In addition, no mutations were detected in the coding regions of *Pfacs10* (PF3D7_0525100) or *Pfacs11* (PF3D7_1238800), which encode acyl-CoA synthetases 10 and 11, respectively. Copy number variation (CNV) analysis revealed a 3.7-fold deamplification in the parasite genome from participant 106 (20 mg of MMV367), involving a 19-kb segment on chromosome 11 spanning three genes: PF3D7_1146700 (putative kinesin-X4), PF3D7_1146800 (putative female development protein FD2), and PF3D7_1146900 (putative protein YIPF6). This CNV, likely reflecting a deletion of these three genes, was not observed in the other drug-exposed parasite genomes (table S4) and was not associated with a change in in vitro susceptibility to MMV367.

Development of a PK/PD model of MMV367

The PK of MMV367 was described by a one-compartment model with linear elimination, first-order absorption without lag time, and a proportional residual error model (table S5). Body weight at baseline was included in the structural model as a covariate using an allometric function with exponents fixed to 0.75 for clearance parameters and 1 for volume parameters. The individual observed MMV367 concentrations were well described by the model (fig. S2). The individual PK parameter estimates were used as regressors in the subsequent PK/PD analysis of MMV367.

The relationship between MMV367 plasma concentrations and parasite killing and clearance was described by a clearance model with a cure threshold (table S6). This model assumes an exponential growth of the parasites and differentiates total and live (viable) parasites, such that the PK drives parasite killing, whereas the clearance of dead parasites is a separate step. Transition of parasites from live to dead was assumed to follow a sigmoid maximum effect (E_{max}) function defined by E_{max}, concentration that leads to 50% of maximum effect (EC_{50}), and Hill parameters. Onward clearance of dead parasites was assumed to be a first-order process. The cure threshold was implemented using a sigmoidal function bound between 0 and 1 that reduces growth asymptotically to 0 when the cure threshold was reached. Given that the model was fit to total parasitemia data (measured by qPCR), E_{max} was fixed to the value and uncertainty interval estimated from the ex vivo viability analysis of MMV367 (mean elimination half-life of viable parasites of 2 hours with MMV367 doses of ≥20 mg) before estimating the other parameters. The individual observed parasitemia profiles were well described by the model (fig. S3). Key efficacy parameters for MMV367 were derived from the PK/PD model (Table 4).

Table 4. Key efficacy parameters for MMV367 derived from the final PK/PD model. Results are based on 500 samples from the estimates' uncertainty distribution (table S3). MIC, minimum inhibitory concentration.

Parameter	Median (95% uncertainty interval)
EC_{50} (ng/ml)	15 (12–18)
E_{max} (1/hour)	0.35 (0.23–0.46)*
PRR_{48}	5.9 (5.7–6)
Parasite killing half-life (hours)	2.5 (2.4–2.5)
MIC (ng/ml)	6.3 (5.2–7.8)
MPC_{90} (ng/ml)	54 (43–68)

*Value and uncertainty interval fixed to ex vivo viability estimate (mean elimination half-life of viable parasites of 2 hours with MMV367 doses of ≥20 mg).

Safety and tolerability

A total of 211 AEs were reported for 11 of 12 participants from malaria inoculation until the end of the study (Table 5), with the majority of these (157 of 211 AEs) being signs and symptoms of malaria. There were no serious AEs. Most AEs were mild (180 of 211 AEs) or moderate (27 of 211 AEs) in intensity. Four AEs were of severe intensity, with three of these being a transient decrease in lymphocyte count attributed to malaria challenge (two events in one participant dosed with 90 mg of MMV367 and one event in one participant dosed with 3 mg of MMV367). The other severe AE was a transient increase in alanine aminotransferase [ALT; 156 U/liter (normal range: 5 to 30 U/liter)] occurring 7 days after one participant (18-year-old female) was dosed with 3 mg of MMV367. This event was considered potentially related to multiple study interventions (malaria challenge, MMV367, artemether-lumefantrine). A concomitant transient elevation in gamma-glutamyl transferase [GGT; 163 U/liter (normal range: 5 to 35 U/liter)], moderate in severity, was observed for this participant.

Higher doses of MMV367 were not associated with an increased incidence or severity of AEs (Table 5). Participants in the lowest dose group (3 mg of MMV367) experienced the highest incidence of AEs, which were predominantly malaria-related events. There were 14 AEs in eight participants that were considered potentially related to MMV367 by the investigator, although 10 of these AEs were also considered potentially related to other study interventions (malaria, definitive antimalarial medications, and other study procedures) because it was not possible to differentiate the exact causality (Table 5). Headache was the only AE considered potentially related to MMV367 that was reported in more than one participant (four events in three participants). The participant dosed with 1500 mg of MMV367 exhibited a T wave inversion (lead V2) on an electrocardiograph (ECG) performed on day 10 (2 days postdose) that spontaneously normalized on day 11. This finding was considered to likely be a benign variant given that T wave inversion confined in V1-V2 is a common finding in the general population (*19*). One 47-year-old male participant experienced a hemoglobin decrease 7 days after being dosed with 90 mg of MMV367 (115 g/liter from 138 g/liter before malaria inoculation). In addition to being considered potentially related to malaria challenge and to MMV367, blood collection for study procedures was considered to have potentially contributed to this event (*20*). Overall, there were no drug-related trends in safety laboratory parameters, vital signs, physical examinations, or ECG parameters over the course of the study.

DISCUSSION

This study aimed to characterize the antimalarial activity of MMV367 by assessing the PK/PD relationship in malaria-naïve adults using the *P. falciparum*–induced blood-stage malaria model. Results confirmed that MMV367 exhibits strong activity against blood-stage *P. falciparum*. The ability of MMV367 to rapidly eliminate viable parasites indicates its potential as an alternative to artemisinins in antimalarial combination therapies.

Single oral doses of ≥20 mg of MMV367 resulted in rapid parasite clearance, with a clearance half-life of about 3 to 4 hours when total parasitemia was measured using qPCR. This clearance half-life is comparable to that of cipargamin (3.99 hours for a single 10-mg dose) (*21*) and faster than that of pyronaridine (5.06 to 5.71 hours for a single dose of 360 to 720 mg) (*15*). However, the proportion of viable parasites (as determined via ex vivo growth) declined at a faster rate after MMV367 dosing in comparison with parasite clearance, with an elimination half-life of about 1 to 2 hours. Similar results have been observed with the artemisinin compound artesunate in the *P. falciparum*–induced blood-stage malaria model, with a single oral dose (2 mg/kg) resulting in a parasite clearance half-life of 3.2 hours (*9*) and a viable parasite elimination half-life of 0.75 hours (*22*). The antimalarial candidate ZY-19489 exhibited a similar profile, with a dose-independent parasite clearance half-life of 6 to 7 hours (*17*) but a dose-dependent viable parasite elimination half-life ranging from 3.7 hours to less than 0.7 hours (*23*). A limitation of the parasite viability analysis is that the estimates assume parasites grow exponentially ex vivo continuously from the time of establishing the cultures until the first detectable parasitemia above 1%. Delayed parasite growth could confound the estimates of parasite viability. However, the validation of the methods in vitro, in mice and in previous human trials (*22*, *24*), indicates that this has not been a major confounder in estimating parasite viability to date but cannot be completely excluded. Further, although the comparable parasite clearance and killing activity of MMV367 and artesunate are encouraging, viable parasite elimination is not a demonstrated surrogate of clinical effect, and further clinical testing is required to establish such a clinical effect.

Lower doses of MMV367 (3, 5, and 10 mg) were intentionally tested in this well-controlled volunteer infection study to optimally characterize the PK/PD relationship before the initiation of clinical trials in patients. These lower doses resulted in parasitemia profiles above the lower limit of quantification of the qPCR assay while also permitting parasite regrowth, thus allowing all PK/PD parameters to be estimated with sufficient certainty. The relationship between MMV367

Table 5. Summary of AEs reported from administration of the malaria challenge agent until the end of the study. The investigator assessed the relatedness of all AEs to malaria challenge, MMV367, and other study interventions (related or unrelated). AEs may have been considered related to more than one study intervention. None of the AEs met the predefined criteria for a serious AE. Data are presented as the number of participants with at least one AE (%); total number of AEs.

AE category	MMV367, 3 mg (*n* = 3)	MMV367, 5 mg (*n* = 2)	MMV367, 10 mg (*n* = 1)	MMV367, 20 mg (*n* = 3)	MMV367, 90 mg (*n* = 2)	MMV367, 1500 mg (*n* = 1)	Total (*n* = 12)
Any AE	3 (100%); 93	2 (100%); 31	1 (100%); 23	2 (66.7%); 29	2 (100%); 29	1 (100%); 6	11 (91.7%); 211
Mild (grade 1)	3 (100%); 81	2 (100); 27	1 (100%); 22	2 (66.7%); 21	2 (100%); 24	1 (100%); 5	11 (91.7%); 180
Moderate (grade 2)	2 (66.7%); 10	2 (100); 4	1 (100%); 1	2 (66.7%); 8	2 (100%); 3	1 (100%); 1	10 (83.3%); 27
Severe (grade 3)	1 (33.3%); 2	0; 0	0; 0	0; 0	2 (100%); 2	0; 0	3 (25%); 4
AE related to malaria	3 (100%); 77	2 (100%); 25	1 (100%); 15	2 (66.7%); 15	2 (100%); 22	1 (100%); 3	11 (91.7%); 157
AE related to MMV367	2 (66.7%); 4	1 (50.0%); 2	0; 0	2 (66.7%); 4	2 (100%); 2	1 (100%); 2	8 (66.7%); 14
Headache*	1 (33.3%); 1	1 (50%); 2	0; 0	0; 0	1 (50%); 1	0	3 (25%); 4
Abdominal pain	0; 0	0; 0	0; 0	1 (33.3%); 1	0; 0	0; 0	1 (8.3%); 1
Diarrhea	0; 0	0; 0	0; 0	1 (33.3%); 1	0; 0	0; 0	1 (8.3%); 1
Nausea	0; 0	0; 0	0; 0	0; 0	0; 0	1 (100); 1	1 (8.3%); 1
Discomfort*	1 (33.3%); 1	0; 0	0; 0	0; 0	0; 0	0; 0	1 (8.3%); 1
Fatigue	0; 0	0; 0	0; 0	1 (33.3%); 1	0; 0	0; 0	1 (8.3%); 1
Tachycardia*	0; 0	0; 0	0; 0	1 (33.3%); 1	0; 0	0; 0	1 (8.3%); 1
ALT increased†	1 (33.3%); 1	0; 0	0; 0	0; 0	0; 0	0; 0	1 (8.3%); 1
GGT increased†	1 (33.3%); 1	0; 0	0; 0	0; 0	0; 0	0; 0	1 (8.3%); 1
ECG T wave inversion*	0; 0	0; 0	0; 0	0; 0	0; 0	1 (100%); 1	1 (8.3%); 1
Hemoglobin decreased‡	0; 0	0; 0	0; 0	0; 0	1 (50%); 1	0; 0	1 (8.3%); 1

*AE was considered to be potentially related to MMV367 and malaria challenge. †AE was considered to be potentially related to MMV367, malaria challenge, and definitive antimalarial medication (artemether-lumefantrine). ‡AE was considered to be potentially related to MMV367, malaria challenge, and a study procedure (blood collection).

plasma concentrations and parasite clearance was best described by a clearance PK/PD model that included a cure threshold. This model is somewhat different from the PK/PD models developed recently for other antimalarials assessed in the *P. falciparum*–induced blood-stage malaria model. The PK/PD models developed for pyronaridine (*15*), tafenoquine (*18*), and ZY-19489 (*17*) used E_{max} PK/PD models that describe total parasites only, whereas the clearance model used here describes live and dead parasites separately. As such, care should be taken when comparing efficacy parameters derived from the PK/PD model in this study with previous induced blood-stage malaria studies. The ability to assess the effect of antimalarials on both total parasites and viable parasites in volunteer infection studies highlights the utility of these studies in characterizing the activity and mode of action of drugs in vivo while also potentially improving the utility of the PK/PD model. The PK/PD model of MMV367 will be valuable in informing dosing strategies and partner drug selection to be used in future trials in patients with malaria.

The PK profile of MMV367 observed in participants with induced blood-stage malaria is broadly consistent with that observed in healthy adults (without malaria challenge) in the first-in-human study (*13*). PK data from both studies were combined for the purpose of developing a population PK model of MMV367 (this model was subsequently used in the PK/PD assessment). Notably, the first two clinical trials of MMV367 used healthy adult populations who were predominantly Caucasian. It will be important to assess the PK profile of MMV367 in patients with malaria in future studies and in infants and pregnant women who represent highly vulnerable groups characterized by physiological changes that affect the PK exposure of various drugs (*25*). The elimination half-life of MMV367 observed in healthy participants (13 to 18 hours) is notably longer than that of artemisinins (typically <1 to 2 hours) (*6*), thus offering the advantage of an extended period at which parasiticidal drug concentrations are maintained after dosing. This property, together with the rapid decrease in viable parasitemia after dosing, may reduce the likelihood of parasite resistance emerging to partner drugs. Furthermore, the longer elimination half-life of MMV367 may enable any future combination therapy with this drug to be potentially administered with a reduced dosing schedule compared with ACTs, which typically require once or twice daily dosing for 3 consecutive days for the treatment of uncomplicated *P. falciparum* malaria.

There was no evidence of emerging parasite drug resistance after in vivo exposure to MMV367 in this study. Whole-genome sequencing confirmed the absence of high-confidence SNP differences between the drug-exposed parasite lines and the untreated parental strain. The MMV367-exposed lines also did not exhibit altered susceptibility to dihydroartemisinin, monodesethyl-amodiaquine, or pyronaridine,

which are key components of current first-line ACTs for uncomplicated *P. falciparum* malaria, each with a distinct mode of action from MMV367. An important limitation of the parasite drug resistance assessment in this study is that the sample size for this analysis was limited ($n = 5$ participants), with parasite lines from four participants exposed to MMV367 for only 2 to 4 days, representing just one or two parasite replication cycles. It will be important to monitor for potential drug resistance development in future trials in larger patient populations.

Gametocytemia was not detected in any participant after administering MMV367. This contrasts with some other drugs tested in the *P. falciparum*–induced blood-stage malaria model, such as pyronaridine (*15*) and piperaquine (*26*), where mature gametocytes were observed entering the circulation after clearance of asexual parasitemia. The apparent inhibition of gametocyte maturation by MMV367 may be related to its rapid parasiticidal activity against asexual stages, preventing sexual stage commitment. It is also possible that MMV367 exhibits activity against early-stage sequestered gametocytes. Further studies to investigate the effect of MMV367 treatment on malaria transmission are needed.

There were no safety concerns associated with single-dose MMV367 administration in this study, which supports the results of the first-in-human study that indicated that MMV367 is well tolerated in healthy adults (*13*). Distinguishing the causality of AEs in volunteer infection studies involving malaria-naïve healthy volunteers can be difficult because of multiple study interventions (malaria challenge agent, investigational drug, and definitive antimalarial medication) and the absence of a control group. Thus, several AEs in this study were considered to be potentially drug related, in addition to being potential signs or symptoms related to malaria challenge. Nevertheless, higher doses of MMV367 were not associated with an increased incidence or severity of AEs overall. Furthermore, there were no clinically concerning trends in any clinical laboratory, ECG, or vital signs parameters. The malaria-related AEs observed were consistent with previous malaria volunteer infection studies. Assessing the safety and tolerability of MMV367 in the target patient population will be an important aim of future trials.

The main limitation of this study was that assessments of MMV367 were performed in a small number of nonimmune healthy adults with experimental malaria infection. There are potential differences in PK and drug tolerability in the target population for treatment, which includes children and pregnant women. The propensity for parasites to develop drug resistance is a very important consideration for the clinical development of promising new antimalarials. The parasite drug resistance assessment performed in this study was limited to drug-exposed parasite populations from only five study participants. Further, these parasite populations were of a relatively low density and were exposed to MMV367 for a short time (up to 4 days in four of the five participants) before definitive artemether-lumefantrine therapy was initiated. Considerably higher parasite densities will be potentially exposed to MMV367 for longer periods of time during clinical use, although combination with one or more partner drugs will mitigate the risk of resistance development. Despite these limitations, data obtained in malaria volunteer infection studies have previously proven useful in guiding future clinical development of several antimalarials (*14*, *16*, *17*, *21*, *27*, *28*). The antimalarial activity characterized in volunteer infection studies in healthy nonimmune volunteers has been found to translate well to patients in endemic areas with clinical malaria (*29*).

This study represents clinical assessment of the antimalarial activity of the pyrrolidinamide antimalarial drug class. Its mode of action, thought to be potentially related to interference with *P. falciparum* acyl-CoA synthetases 10 and 11, supports the potential of this drug class to combat the loss of efficacy of existing antimalarials and reduce malaria morbidity and mortality. This volunteer infection study has confirmed that the pyrrolidinamide MMV367 is well tolerated in healthy participants at doses that rapidly kill blood-stage *P. falciparum*. Notably, similar to artemisinins, parasite killing kinetics are faster than clearance kinetics, suggesting that MMV367 exerts an irreversible parasite effect making it an attractive candidate for further development. The results of this study support the continued clinical development of MMV367 as a component of a treatment for uncomplicated *P. falciparum* malaria.

MATERIALS AND METHODS

Study design

This was an open label, randomized, clinical trial using the induced blood-stage malaria model to characterize the antimalarial activity of MMV367. Healthy malaria-naïve adults (nonpregnant, nonlactating), including women of childbearing potential, aged 18 to 55 years were eligible for inclusion (see the Supplementary Materials). The study was conducted at the University of the Sunshine Coast Clinical Trials Units (Morayfield and South Brisbane, Australia) and was registered on ClinicalTrials.gov (NCT05979207). This study was approved by the QIMR Berghofer Human Research Ethics Committee (P3879). All participants gave written informed consent before enrollment. Participants were randomized within each cohort to a MMV367 dose group on day 8, following the establishment of parasitemia. A paper-based randomization list was generated manually. No masking was performed.

The primary outcome was the PK/PD relationship of MMV367. Secondary outcomes were parasite clearance kinetics parameters, MMV367 noncompartmental PK parameters, and the safety and tolerability profile of MMV367 and malaria challenge. A maximum sample size of up to 18 participants, enrolled in sequential cohorts of up to six participants each, was expected to be sufficient to achieve the primary outcome of the study. This sample size was not based on formal statistical calculations but rather was based on previous experience of characterizing the PK/PD relationship of antimalarials using the induced blood-stage malaria model (sample sizes ranged between 9 and 15 participants) (*15*, *17*, *18*). The specific number of participants required was dependent on the emerging results during the study. The Safety and Data Review Team reviewed all data (including preliminary PK/PD modeling) after the completion of each cohort and decided when sufficient data had been obtained to complete the primary end point.

Procedures

Participants were inoculated intravenously with about 2800 viable *P. falciparum* 3D7-A–infected erythrocytes on day 0. Parasitemia was monitored throughout the study by qPCR targeting the gene encoding *P. falciparum* 18*S* ribosomal RNA (rRNA) (*30*). A single dose of MMV367 (Piramal Pharma Solutions, Mumbai, India) was administered as an aqueous oral suspension on day 8 after an overnight fast. Participants remained within the clinic for 72 hours postdose for frequent safety assessments and blood collection. Follow-up outpatient visits then occurred up to day 27. Participants received

a curative course of artemether-lumefantrine (Riamet, Novartis Pharmaceuticals) on day 24 or earlier in response to insufficient parasite clearance following MMV367 dosing, parasite regrowth, clinical symptoms of malaria, or at the discretion of the investigator.

Doses of MMV367 were selected to optimally characterize the PK/PD relationship, which included the testing of lower doses expected to result in "suboptimal" parasite clearance. The doses tested in cohort 1 (20, 90, and 1500 mg) were based on preliminary modeling using human PK data generated in the first-in-human study (*13*) combined with preclinical PD data (humanized mouse model of malaria and in vitro killing rates). A single dose of 20 mg was expected to result in initial parasite clearance in the induced blood stage malaria model {$\log_{10}$ parasite reduction rate over 48 hours (PRR_{48}) median 2.9 [95% confidence interval (CI), 0.26 to 5.8]}, but subsequent parasite regrowth was expected to occur. A dose of 90 mg was predicted to represent an "intermediate" dose, with faster parasite clearance [$\log_{10}$ PRR_{48} median 3.6 (95% CI, 1.8 to 6.2)] compared with 20 mg. Last, testing a dose of 1500 mg (the highest dose previously tested and found to be well tolerated in the first-in-human study) was required to characterize the PK/PD parameters across the full drug concentration range, particularly the maximum killing rate.

The doses for cohort 2 (3, 5, and 10 mg) were informed by a preliminary PK/PD model, which used data from the preceding cohort and were selected to refine the PK/PD parameter estimates. Specifically, the data obtained from cohort 1 indicated that it was necessary to test doses lower than 20 mg in cohort 2 to improve the estimation of PK/PD parameters such as the EC_{50}. That is, lower doses were required to increase the chances of parasite regrowth and of achieving a full parasitemia profile above the lower limit of quantification of the qPCR assay.

Plasma concentrations of MMV367 were measured by liquid chromatography tandem mass spectrometry using a validated method (see the Supplementary Materials). The presence of female gametocytes, male gametocytes, and ring stage parasites in blood samples was assessed from day 12 using quantitative reverse transcription PCR (qRT-PCR) targeting *pfs25*, *pfMGET*, and *SBP-1* parasite transcripts, respectively (*31*, *32*). Safety assessments—including monitoring of AEs, vital signs, hematology and biochemistry, physical examination, and electrocardiographs—were performed throughout the study.

Quantifying parasite growth ex vivo was used to measure parasite viability since the 18*S* qPCR assay quantifies all parasites (including those nonviable or dead in circulation). Ex vivo parasite cultures were established from freshly sampled blood collected before MMV367 administration, at 8, 16, 24, 36, 48, 72, and 96 hours after MMV367 administration, and immediately before definitive antimalarial treatment (see the Supplementary Materials). The percentage of infected red blood cells (parasitemia) in ex vivo cultures was determined using flow cytometry daily for up to 30 days or until parasitemia was ≥3%. Parasite growth in cultures was analyzed using a mathematical model to estimate the viable parasite concentration in each blood sample (see the Supplementary Materials) (*22*, *24*).

Blood samples were collected from participants with parasitemia immediately before definitive antimalarial treatment to investigate the emergence of parasite drug resistance following exposure to MMV367. Parasite samples were initially cryopreserved and then cultured in vitro for 8 to 14 days to reach 2 to 6% parasitemia. The 50% (IC_{50}) and 90% (IC_{90}) growth-inhibitory MMV367 concentrations for drug-exposed parasite lines and for the drug-sensitive 3D7-A parental strain were determined using in vitro 72-hour growth inhibition assays (see the Supplementary Materials). MMV367-exposed parasites were also assessed for susceptibility to dihydroartemisinin, the active amodiaquine metabolite monodesethyl-amodiaquine, and pyronaridine, antimalarial drugs with distinct modes of action that are in clinical use. Whole-genome sequencing was performed on genomic DNA extracted from drug-exposed parasite samples and the 3D7-A parental strain using 300–base pair paired-end, dual index Illumina libraries to identify genetic determinants associated with resistance to MMV367 (see the Supplementary Materials).

Statistical analysis

PK and PK/PD modeling—including data processing, model setup, and modeling result analysis—were conducted within R (3.6.3) using the IQR tools package (1.9.0) developed by IntiQuan (Basel, Switzerland) and the MMVmalaria package (1.0.0) developed by Medicines for Malaria Venture (MMV; Geneva, Switzerland). A nonlinear mixed effects modeling approach using the stochastic approximation expectation maximization method of Monolix (MLX2019R1) was used (see the Supplementary Materials). A population PK model was developed using PK data from the current study in conjunction with PK data from the first-in-human study of MMV367 (table S7) (*13*) to obtain individual PK parameter estimates that adequately described the observed individual PK profiles. Model selection was based on the Bayesian Information Criterion, precision of parameter estimates, goodness of fit and visual predictive check plots, and visual evaluation of the individual plasma concentration profiles. The PK/PD model was then built using the individual PK parameter estimates as regression parameters. Parasitemia data were log-transformed, and observations below the lower limit of quantification were censored. The precision of parameter estimates, visual evaluation of the individual parasitemia profiles, and goodness of fit plots ensured that the data were adequately described by the final PK/PD model. Key efficacy parameters including the EC_{50}, E_{max}, PRR_{48}, parasite elimination half-life, minimum inhibitory concentration, and minimum parasiticidal concentration (concentration that leads to 90% of maximum effect) (MPC_{90}) were derived from the final PK/PD model (see the Supplementary Materials).

The analysis of parasite clearance kinetics following MMV367 dosing was performed in R statistical package version 4.3.1. 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 (*33*). Noncompartmental PK analysis was performed using Phoenix WinNonlin version 8.3. The incidence of parasite drug resistance and the effect of MMV367 on parasite viability were exploratory outcomes.

Supplementary Materials

The PDF file includes:
Materials and Methods
Figs. S1 to S3
Tables S1 to S7
Reference (*34*)

Other Supplementary Material for this manuscript includes the following:
MDAR Reproducibility Checklist
CONSORT Checklist

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The pyrrolidinamide antimalarial drug MMV367 rapidly clears blood-stage *Plasmodium falciparum* in healthy adults with experimental malaria

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Editor's summary

The pyrrolidinamide drug MMV367 has been tested preclinically as an antimalarial. Barber *et al.* now report a clinical assessment of the antimalarial activity of MMV367 in healthy volunteers experimentally infected with blood-stage *Plasmodium falciparum*, the protozoan parasite that causes malaria. Twelve experimentally infected volunteers were randomized to receive single-dose oral administration of MMV367. MMV367 was well tolerated by volunteers and rapidly killed blood-stage malaria parasites. These findings support the continued development of MMV367 as a treatment for malaria. —Orla Smith