Safety, tolerability, pharmacokinetics and antimalarial activity of the novel Plasmodium phosphatidylinositol 4-kinase inhibitor MMV390048 in healthy volunteers

Running title: MMV390048 safety, PK and antimalarial activity

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ABSTRACT

MMV390048 is a novel antimalarial compound that inhibits *Plasmodium* phosphatidylinositol-4-kinase. The safety, tolerability, pharmacokinetic profile, and antimalarial activity of MMV390048 were determined in healthy volunteers in three separate studies. A first-in-human, double-blind, randomized, placebo-controlled, single ascending dose study was performed. Additionally, a volunteer infection study investigated the antimalarial activity of MMV390048 using the *P. falciparum* induced blood stage malaria (IBSM) model. Due to the high pharmacokinetic variability with the powder-in-bottle formulation used in both these studies, a third study was undertaken to select a tablet formulation of MMV390048 to take forward into future studies. MMV390048 was generally well tolerated when administered as a single oral dose up to 120 mg, with rapid absorption and a long elimination half-life. Twelve adverse events were considered to be potentially related to MMV390048 in the first-in-human study, but with no obvious correlation between these and MMV390048 dose or exposure. Although antimalarial activity was evident in the IBSM study, rapid recrudescence occurred in most subjects after treatment with 20 mg MMV390048, a dose expected to be sub-therapeutic. Reformulation of MMV390048 into two tablet formulations (tartaric acid and syloid) resulted in significantly reduced inter-subject pharmacokinetic variability. Overall, the results of this study suggest that MMV390048 is well tolerated in humans and the pharmacokinetic properties of the compound indicate that it has the potential to be used for antimalarial prophylaxis or inclusion in a single-dose cure. MMV390048 is currently being tested in a phase 2a study in Ethiopian adults with acute, uncomplicated falciparum or vivax malaria mono-infection.

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Keywords: Malaria, *Plasmodium falciparum*, MMV390048, first-in-human, safety, pharmacokinetics, volunteer infection study, phosphatidylinositol-4-kinase

Clinical trials registration: The three clinical trials described were each registered with ClinicalTrials.gov (first-in-human study, NCT02230579; IBSM study, NCT02281344; formulation optimisation study, NCT02554799).

INTRODUCTION

Loss of efficacy of key artemisinin-based combination therapies in the Greater Mekong subregion highlights the urgent need for novel antimalarials (1). Resistance is now widely prevalent across at least six countries to both the fast-acting artemisinins and to their partner drugs (2). Drug resistance is a major threat to achieving malarial control and elimination goals as articulated by the World Health Organization (3). New antimalarials with novel mechanisms of action are urgently needed to combat parasite drug resistance and reduce malaria morbidity and mortality.

Several antimalarial candidates are currently in development (4, 5). One of these is MMV390048, an aminopyridine discovered through optimisation of a high-throughput screening hit compound (6). MMV390048 selectively inhibits *Plasmodium* phosphatidylinositol-4-kinase (PI4K) (7), which is an attractive target for antimalarial drug development because it is required across all *Plasmodium* lifecycle stages (8). Preclinical studies predicted a long elimination half-life of ~90 hours in humans, good oral bioavailability, and that a single oral dose of 80–100 mg
would maintain the concentration of MMV390048 above therapeutic levels for 8 days – a
duration estimated to result in cure (approximately 4 asexual *P. falciparum* life-cycles) (9).

Overall, results from preclinical studies indicated that MMV390048 has potential for inclusion in
a single-dose antimalarial therapy (10, 11), and supported progression of the compound to
clinical development.

In this report, we present results from three phase 1 clinical studies of MMV390048. The safety,
tolerability and pharmacokinetic profile of MMV390048 in a powder-in-bottle formulation were
assessed in a first-in-human clinical trial. The antimalarial activity of the MMV390048 powder-
in-bottle formulation was also tested in a volunteer infection study using the induced blood stage
malaria (IBSM) model, whereby healthy subjects are inoculated with blood stage *P. falciparum*.

Finally, the pharmacokinetic profiles of two MMV390048 tablet formulations were assessed in a
formulation optimisation study.

**RESULTS**

**Study subjects**

The subject flow for the three studies is presented in Figure 1, and subject demographics are
summarised in Table 1. Forty subjects were enrolled in the first-in-human study in five
sequential, fasted dose cohorts (5 mg, 20 mg, 40 mg, 80 mg or 120 mg). Within each cohort,
subjects were dosed with either MMV390048 powder-in-bottle formulation (6 subjects) or
placebo (2 subjects). A sixth dose cohort (40 mg FED) was dosed under fed conditions and was
composed of subjects who had previously received treatment as part of the 5 mg or 40 mg fasted
dose cohorts (Figure 1). The majority of subjects were male (36/42; 86%) and most self-declared
their ethnic identity as black (31/42; 74%) (Table 1). One subject (120 mg dose cohort) used undisclosed prohibited prior medication (carbamazepine and an unknown traditional Chinese treatment) for an undisclosed neurological disease (see safety results section for more details). It was retrospectively confirmed that this subject took carbamazepine until admission. Pharmacokinetic data from this subject were therefore excluded from analyses.

A total of 6 subjects were enrolled in the IBSM study in a single dose cohort (Figure 1). The planned sample size of 8 subjects per dose cohort was not achieved due to recruitment limitations. All 6 subjects were inoculated with blood-stage *P. falciparum* and dosed with 20 mg MMV390048 powder-in-bottle formulation (Figure 1). All subjects were male and Caucasian (Table 1).

Eighteen subjects were enrolled in the formulation optimisation study and were dosed with 40 mg MMV390048 in either a tartaric acid (formulation A; n = 9) or syloid (formulation B; n = 9) tablet formulation (Figure 1). All subjects were male and most were Caucasian (Table 1).

**Safety**

A summary of AEs recorded for each study is presented in Table 2 and a detailed list is included in Table S4, Table S5 and Table S6.

**First-in-human study**

A total of 127 AEs were reported, which were predominantly mild in severity (99/127 [78%]). The incidence and severity of AEs was similar between treatment groups. Fifteen AEs reported
in 13 subjects were considered to be related to the investigational product; three of these AEs occurred in two subjects who received placebo. There was no apparent relationship between the MMV390048 dose or exposure and the incidence or severity of AEs considered treatment related. No trends in inhibin B, FSH, LH or testosterone levels were identified over the course of the study across the different dose groups (Figure S1).

The only serious AE (SAE) reported in this study was generalised myoclonus approximately 24 h after dosing with 120 mg MMV390048, which occurred in a subject with an undisclosed history of epilepsy. This episode resolved after treatment with valproate and clonazepam. An electroencephalogram at the time showed a pattern consistent with a primary generalised form of epilepsy, such as Juvenile Myoclonic Epilepsy. Although the subject had denied any past medical history or any medication use repeatedly, a subsequent search of the subject’s medical records disclosed a 7-year history of poorly-controlled epilepsy and generalised cerebral atrophy, with approximately monthly episodes of generalised myoclonus. This subject had commenced treatment with carbamazepine in November 2013 but had interrupted treatment to enrol into the study. The principal investigator and neurologist consulted assessed the SAE as a pre-existing condition, with the generalised myoclonus probably related to sleep deprivation at the time, and possibly exacerbated by a sub-therapeutic level of carbamazepine, and possibly MMV390048.

Other AEs considered related to MMV390048 were PR prolongation detected on ECG, decrease in blood pressure (to 87/52 mmHg), dizziness, photophobia, malaise, dysgeusia that were all mild in severity, diarrhoea (2 cases in 2 subjects) and papular urticaria that were both moderate in severity, and severe neutropenia. The severe neutropenia (2 events in one subject) occurred
one day after dosing with 40 mg MMV390048 in the fasted and then fed state. This subject, self-identified as a black African, had a non-clinically significant decreased neutrophil count at screening (1.85×10⁹ L⁻¹) and before dosing on both occasions (1.65×10⁹ L⁻¹ and 1.90×10⁹ L⁻¹ for fasted and fed states respectively), consistent with benign ethnic neutropenia (12). The lowest observed neutrophil counts were observed 24 h after dosing (fasted state: 0.77×10⁹ L⁻¹; fed state: 0.97×10⁹ L⁻¹). Neutrophil counts returned to a level >1.5×10⁹ L⁻¹ 4 days (fasted) and 13 days (fed) after MMV390048 dosing.

The case of moderate papular urticaria (bilateral on upper arms) occurred 14 days after dosing with 20 mg MMV390048 and was not associated with eosinophilia or other clinically significant abnormalities. The episode lasted 20 days and responded to treatment with topical hydrocortisone. The case of mild PR prolongation (maximum 285 msec) occurred 24 h after dosing with 20 mg MMV390048, and was still ongoing at the end-of-study follow-up visit (Day 79) despite negligible plasma concentrations of MMV390048 at this point. The subject had a PR-interval of 200.3 msec upon admission to the unit the day prior to MMV390048 dosing. No QT prolongation (QTcF>450 msec) was recorded for any subject during the study, and no other trends or concerns with respect to ECG results were observed.

**IBSM study**

A total of 32 AEs were reported in 5 of the 6 subjects which were predominantly mild in severity (23/32 [71.9%]). The most common AE was headache (7 cases in 4 subjects). Most AEs were deemed related to malaria (81.3%); none were considered related to MMV390048. There were
no SAEs or severe AEs reported and no clinically significant abnormal laboratory safety parameters or ECG findings.

Formulation optimisation study
A total of 11 AEs were reported in 7 of the 18 subjects. There was one case of upper respiratory infection that was of moderate intensity (grade 2); all other AEs were mild. No SAEs were reported. Two AEs were deemed related to MMV390048, both reported in subjects in the syloid-tablet cohort; one was a headache occurring ~7 h after dosing, which resolved within 3 h without treatment; the other was a symmetrical, raised, pruritic, cutaneous rash over the medial aspect of both knees, which occurred 3 days after dosing and resolved spontaneously within 1 h. No clinically significant changes from baseline were seen in biochemistry, haematology, coagulation, urinalysis, vital signs, or ECG parameters.

Pharmacokinetics
In the first-in-human study, peak and total plasma exposures of MMV390048 generally appeared to increase with increasing doses (Figure 2). However, considerable inter-subject variability within each cohort was seen for all pharmacokinetic parameters (Table 3 and Figure S2). The 20 mg cohort showed the least inter-subject variability, but a disproportionally high exposure with respect to other dose cohorts. The elimination half-life of MMV390048 (>150 h) was longer than was predicted from preclinical studies (90 h). Median t\text{max} was 1 to 2.5 h after dosing for the fasted cohorts, but was longer for the 40 mg fed cohort (4 h). Furthermore, administration of a FDA prescribed high fat breakfast (13) prior to dosing reduced inter-subject variability for all pharmacokinetic parameters in comparison to the equivalent dose administered fasted, although
variability was still moderate. Investigations were performed to assess whether any manufacturer or site factors explained or contributed to the high levels of inter-individual variability observed. It was concluded that neither dosing nor packaging or preparation of MMV390048 were likely to be the source of variability.

High inter-subject variability was also observed in the pharmacokinetic parameters in the IBSM study (Table 3 and Figure S3). MMV390048 plasma concentrations were only assayed in samples taken up to 192 h after MMV390048 dosing (Figure 2). The remainder of samples were not analysed due to the significant variability observed, and the sponsor decision to suspend the study to reformulate the compound.

Absorption of MMV390048 after ingestion of the two tablet formulations was rapid following dosing, with a median $t_{\text{max}}$ of 3 h for both formulations (Table 3 and Figure S3). $C_{\text{max}}$ and $AUC_{0-\text{inf}}$ were higher in the syloid-tablet (368.3 ng mL$^{-1}$ and 57 608 ng·h mL$^{-1}$, respectively) than in the tartaric acid-tablet (271.0 ng mL$^{-1}$ and 49 726 ng·h mL$^{-1}$, respectively). For both formulations, inter-individual variability was relatively low for $C_{\text{max}}$ (CV 18.0–22.3%), and moderate for $AUC_{0-\text{inf}}$ (CV 48.6–53.5%). The geometric mean $t_{1/2}$ was longer for the tartaric acid-tablet (179 h) than for the syloid-tablet (149 h), although variability was similar (CV 37.6–44.0%).

Antimalarial activity in the *P. falciparum* induced blood stage malaria model
Parasitemia decreased initially in all subjects following administration of 20 mg MMV390048 (Figure 3). However, recrudescence was observed in all subjects either 2 days (n=4) or 7 days (n=2) after MMV390048 dosing. The response to MMV390048 was prolonged in the two
subjects who had the highest exposure; the area under the concentration-time curve from 0 h to
the last time point measured (AUC$_{0\text{-last}}$) was approximately 12 μg·h mL$^{-1}$ for the two subjects
who recrudesced 7 days after treatment compared with approximately 6-9 μg·h mL$^{-1}$ for the 4
subjects who recrudesced 2 days after treatment (data not shown). Subjects were treated per
protocol with artemether-lumefantrine within 24 h of recrudescence. One subject showed
evidence of gametocytemia at the time of artemether-lumefantrine treatment so was also treated
with a single dose of 45 mg primaquine. All subjects were aparasitemic by the end of the study
(Day 28). Pharmacodynamic assessment of parasite clearance after MMV390048 treatment was
not performed due to the smaller than planned sample size as a result of only one dose cohort
being enrolled, rapid recrudescence, and the high inter-subject pharmacokinetic variability
associated with the powder-in-bottle formulation.

**DISCUSSION**

In this report, we present the safety, pharmacokinetic profile and antimalarial activity of
MMV390048, the first *Plasmodium* PI4K inhibitor to reach clinical development. First-in-human
studies are not typically conducted in low- and middle-income countries due to limitations in
technical expertise and infrastructure (14). The successful completion of the MMV390048 first-
in-human study at the University of Cape Town represents an important advance in this respect.

Overall, the results of the three clinical trials indicate that MMV390048 is generally well
tolerated in healthy adult subjects up to a single oral dose of 120 mg. One SAE, generalized
myoclonus, was reported in a subject with an undisclosed history of epilepsy. The subject’s
cessation of his prescribed anticonvulsants and sleep deprivation were the most likely factors
causing his seizure, although the potential of MMV390048 to lower the seizure threshold could not be excluded.

Severe neutropenia was observed in one subject on both occasions when dosed with 40 mg MMV390048 (once in fasted, once in fed state). Although this subject was felt to have benign ethnic neutropenia (12), and no clinically significant abnormal neutrophil values were recorded in the IBSM study or formulation optimisation study, neutrophil counts should be carefully monitored in future trials. All other AEs considered potentially related to MMV390048 were mild or moderate in severity, with no obvious correlation between the incidence or severity of these AEs and the dose or exposure of MMV390048. Additionally, other than diarrhoea, which was reported for 2 subjects, all other AEs related to MMV390048 were observed in a single subject only. Collectively, these data support the further clinical development of MMV390048 for antimalarial prophylaxis or treatment.

The pharmacokinetic profiles of MMV390048 varied considerably between subjects within the same dose-cohort in both the first-in-human and IBSM studies, where the powder-in-bottle formulation was used. Peak and total exposures generally increased with increasing doses (except the 20 mg cohort in the first-in-human study), but dose-exposure was difficult to interpret, and linearity could not be established. Estimates of elimination half-life were highly variable within each cohort resulting in significant uncertainty regarding the actual half-life of MMV390048. The variability was considered to be likely due to differential absorption of the powder-in-bottle formulation of MMV390048 between individual subjects rather than dosing, packaging or preparation of the compound at either site.
The IBSM study started after the 5 mg and 20 mg dose cohorts of the first-in-human study were completed. Although preclinical work indicated that MMV390048 is active against all *Plasmodium* life cycle stages (9) and thus has the potential to target liver stage infection, the current study focused on determining its activity against blood stage parasites as these cause the clinical features of malaria. An attractive characteristic of the IBSM model is that it can be conducted in parallel with an ongoing first-in-human study, allowing accrual of key pharmacokinetic and pharmacodynamic data in a short time frame. This enables rapid prediction of the potential clinically efficacious dose, and hastens the process of drug development. Although there was evidence of antimalarial activity in the IBSM study, and notably that recrudescence was delayed in the two subjects with higher MMV390048 exposure, the high variability in exposure and response prevented progression to the evaluation of the 80-100 mg dose expected to be effective and the execution of the planned pharmacokinetic and pharmacodynamic modelling. Instead it was decided to reformulate the compound.

A powder-in-bottle formulation was chosen for the first-in-human and IBSM studies since it represents a cost and time effective strategy to progress a compound from preclinical to clinical studies. Despite the pharmacokinetic variability observed with the powder-in-bottle formulation of MMV390048, the studies using this formulation led to confirmation that the compound was well tolerated and had antimalarial activity in humans and thus reduced the risk associated with investing in more sophisticated formulation work. Two tablet formulations (tartaric acid and sylloid) were selected from various tablet formulation prototypes based on their stability and dissolution performance in biorelevant media. Both tablet formulations exhibited substantially
reduced variability in exposure and other pharmacokinetic parameter estimates, anda long
elimination half-life that was greater than predicted in preclinical studies (90 h). The tartaric acid
formulation was chosen to be progressed in further clinical trials due to its favourable
pharmacokinetic profile and less challenging manufacturing process compared with the syloid
formulation. Results from the fed cohort in the first-in-human study suggested that food intake
may delay absorption of MMV390048, but this needs to be confirmed by repeating a food effect
study with the final clinical formulation.

Despite the significant pharmacokinetic variability observed with the powder-in-bottle
formulation, the results reported here support the potential for MMV390048 to be included in a
single-dose cure combination regimen (or chemoprophylaxis) for falciparum malaria. The
preclinical efficacy study conducted in humanised severe combined immunodeficient mice
infected with *P. falciparum* predicted an effective concentration to achieve 90% of the maximal
kill rate (EC$_{90}$) of 160 ng mL$^{-1}$ (9). The long elimination half-life for MMV390048 in humans
was confirmed in the clinical studies and the geometric mean MMV390048 plasma concentration
in the 120 mg dose cohort (first-in-human study) remained above the EC$_{90}$ for 6 days after
dosing, despite the high variability of exposure with the powder-in-bottle formulation. Thus,
parasiticidal concentrations of MMV390048 can be maintained for an extended period after
administration of a single dose, potentially preventing recrudescence without the need for a
multiple dose treatment regimen. A follow up IBSM study to characterise the pharmacodynamic
antimalarial activity of the MMV390048 tablet formulation and to estimate the efficacious dose
in humans has recently been completed and the results will be published separately.
In summary, MMV390048 was well tolerated when administered as a single oral dose up to 120 mg in healthy volunteers and displayed reduced pharmacokinetic variability after reformulation of the powder-in-bottle to a tablet formulation. This antimalarial candidate has the potential to be used for prophylaxis or inclusion in a single-dose cure. MMV390048 is currently being tested in a phase 2a study in Ethiopian adults with acute, uncomplicated falciparum or vivax malaria mono-infection (ClinicalTrials.gov Identifier: NCT02880241).

MATERIALS AND METHODS

Clinical studies design

The first-in-human study was conducted at the University of Cape Town Clinical Research Centre (Cape Town, South Africa) between 30 April 2014 and 2 February 2015. This was a phase 1, adaptive, single centre, double-blind, randomized, placebo-controlled, ascending dose study. The study was planned in two parts: a single ascending dose, and a multiple ascending dose. However, the multiple ascending dose part was not executed due to the high variability in pharmacokinetic profiles observed during the single ascending dose part (see results section).

The second study was conducted at Q-Pharm (Brisbane, Australia) from 27 October 2014 to 19 December 2014. This was an IBSM, phase 1b, single centre, open-label study, in which a single dose of MMV390048 was administered to subjects previously inoculated with blood stage P. falciparum. This study was planned to consist of two dose cohorts, however a second cohort was not enrolled due to the high variability in pharmacokinetic profiles observed in the first cohort and in the first-in-human study (see results section). The IBSM study commenced when the first
two dose cohorts of the first-in-human study had been completed. MMV390048 was administered in the first-in-human and IBSM studies in a powder-in-bottle formulation.

The third study was a formulation optimisation study to test two MMV390048 tablet formulations (tartaric acid and syloid). The formulation optimisation study was conducted at Richmond Pharmacology Ltd (London, United Kingdom) from 17 September to 28 October 2015. This was a phase I, open-label, single-dose, parallel group, two cohort design study.

Healthy subjects aged 18 to 55 years were eligible for the studies (first-in-human study and formulation optimisation study: men and women of non-childbearing potential; IBSM study: malaria naïve men only). A complete list of the inclusion and exclusion criteria for each study is included in the supplementary file.

The first-in-human study was approved by the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee (approval number 009/2014). The IBSM study was approved by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee (approval number H0509-046T [P922]). The formulation optimisation study was approved by the National Research Ethics Service Committee London-Brent (approval number 15/LO/1415). The studies were registered with ClinicalTrials.gov (first-in-human study, NCT02230579; IBSM study, NCT02281344; formulation optimisation study, NCT02554799). All studies were conducted according to the Declaration of Helsinki and the ICH Guideline for Good Clinical Practice. All subjects gave written, informed consent before screening.
Randomization and blinding

Subjects in the first-in-human study were randomized to either MMV390048 or placebo in a 3:1 ratio. Treatment identity was concealed by identical packaging and appearance, odour and taste of both MMV390048 and placebo. Subjects in the two parallel cohorts of the formulation optimisation study were randomized in a 1:1 ratio to one of the two MMV390048 tablet formulations. The randomization schedules were generated electronically by independent, unblinded statisticians using the PROC PLAN procedure in SAS®. No randomization was performed in the IBSM study.

Procedures

First-in-human study

The study was conducted in five fasted cohorts (5, 20, 40, 80 and 120 mg) and one fed 40 mg cohort. The initial dose of 5 mg was chosen in agreement with guidelines on dose selection for first-in-human clinical studies (15, 16), and was based on the No Observed Adverse Event Level for the most sensitive species observed in preclinical toxicology studies. Doses administered to subsequent cohorts were determined \textit{a priori} after consideration of interim human pharmacokinetic and safety data.

MMV390048 and placebo were each supplied as a powder for suspension packaged in white opaque high-density polyethylene bottles, with a volume capacity of 100 mL when reconstituted with deionized water (Almac Pharma Services, United Kingdom). The powder contained the following excipients: Avicel CL611, disodium edetate, sodium citrate dihydrate, methyl paraben,
sucralose, povidone and orange flavour. The reconstituted formulation consisted of the active
ingredient MMV390048 in an aqueous suspension of 5 mg mL\(^{-1}\).

All subjects in the fasted cohorts were dosed on Day 1 after a fasting period ≥8 h. Water was
prohibited from 2 hours pre-dose and then allowed \textit{ad libitum} from 2 hours post-dose and
standardised meals were provided from 4 hours post-dose. In the first two cohorts (5 and 20 mg),
a sentinel sub-cohort (\(n = 2\)) was dosed on Day 1. The safety review team reviewed safety and
pharmacokinetic data of these subjects up to Day 3 before dosing the rest of these two cohorts,
and up to Day 19 for all cohorts before dose escalation. Subjects in the 5 mg cohort were
followed until Day 29 while subjects in subsequent cohorts were followed until Day 77 as the
elimination half-life of MMV390048 in Cohort 1 was found to be longer than predicted from
preclinical data. Selected subjects from the fasted cohorts then received 40 mg in a fed state to
evaluate the effect of food on the tolerability and pharmacokinetic profile of MMV390048.

Subjects in the fed cohort had a high fat breakfast 30 min before dosing, which occurred at least
five half-lives after the fasted dose to ensure minimal carry-over effect. The pre-dose breakfast
was in line with FDA guidelines (13) and was composed of approximately 800-1000 calories
with 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.

\textit{IBSM study}

The dose of MMV390048 chosen (20 mg) was based on the pharmacokinetic, safety and
tolerability data available from the first-in-human study at the time the IBSM study commenced
(when 5 and 20 mg dose cohorts of the first-in-human study had been completed). This dose was
below the estimated single dose required to achieve complete cure calculated from preclinical
studies (80-100 mg) since recrudescence allows for characterization of the pharmacokinetic/pharmacodynamic relationship between MMV390048 and *P. falciparum* parasitemia. Subjects were inoculated intravenously on Day 0 with erythrocytes infected with ~1800 viable chloroquine-sensitive *P. falciparum* 3D7 parasites (17). Fasted subjects (≥8 h) were dosed with MMV390048 when they reached the parasitemia threshold for treatment (~1000 parasites mL⁻¹), and were followed up until the end of study visit on Day 28.

**Formulation optimisation study**

Subjects received one of two tablet formulations (formulation A: tartaric acid tablets, formulation B: syloid tablets; manufactured by Quotient Clinical, United Kingdom) on Day 1, after ≥8 h fasting period, at a dose of 40 mg with 240 mL of water. Subjects were followed up until Day 29.

**Safety**

Safety assessments were performed at protocol-specified times (the full schedule of events for each study is presented in Table S1, Table S2, and Table S3). Safety endpoints were the incidence, severity and relationship to the investigational product (and inoculum for the IBSM study) of observed and self-reported adverse events (AEs); and changes from baseline in physical examination, vital signs, standard electrocardiograms (ECGs), continuous ECGs (first-in-human study only), and laboratory evaluation (haematology, coagulation parameters, haemolysis panel, clinical chemistry, and urinalysis) findings. Additionally, inhibin B, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were measured for endocrine assessment of testicular function in the first-in-human study since testicular toxicity.
was observed in pre-clinical toxicology studies conducted in rats. AE severity was evaluated according to the Common Terminology Criteria for Adverse Events version 4.03 (18) and the World Health Organisation recommendations for grading acute and subacute toxic effects (19).

**Pharmacokinetics**

Blood samples were collected at the following time intervals after dosing to determine the MMV390048 plasma concentration and calculate pharmacokinetic parameters. First-in-human study: 0.5, 1, 2, 3, 4, 6, 8, 9, 12, 24, 48, 96, 144, 216, 312, 432, 600, 672 hours for the 5 mg cohort; and additional 816 (Day 35), 1152 (Day 49), 1488 (Day 63), and 1824 (Day 77) hours for the subsequent cohorts. IBSM study: 0.5, 1, 2, 3, 4, 6, 8, 12, 14, 16, 20, 24, 30, 48, 72, 96, 144, 192, 240, 336, 432, 505 hours. Formulation optimisation study: 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 144, 216, 312, 432, 600, 672 hours.

Blood samples of the three studies were analysed at the University of Cape Town, Division of Clinical Pharmacology Laboratory. MMV390048 was extracted from plasma using a protein precipitation procedure, and analysed using a liquid chromatography tandem mass spectrometry assay. The precision (total-assay coefficients of variation) for MMV390048 during sample analysis was less than 8% at all quality control levels, including the limit of quantification, which was 0.5 ng mL⁻¹. Full details of the assay are included in the supplementary file.

Non-compartmental pharmacokinetic analysis was performed using Phoenix® WinNonlin® Pro version 6.3 (Certara L.P., St. Louis, MO, USA) for the first-in-human and the formulation optimisation studies, and using R version 3.2.0 for the IBSM study. Pharmacokinetic endpoints
were the peak plasma concentration (C<sub>max</sub>), time at which C<sub>max</sub> is reached (t<sub>max</sub>), area under the concentration-time curve from 0 h to infinity (AUC<sub>0-inf</sub>), elimination half-life (t<sub>1/2</sub>), apparent clearance (CL/F), and apparent volume of distribution (Vz/F).

Parasitemia measurement

Parasitemia was monitored by collecting blood samples and performing quantitative PCR (qPCR) targeting the gene encoding P. falciparum 18S rRNA (20). Blood samples were collected daily from Day 4 until parasites were detectable; then twice daily until MMV390048 dosing; then before MMV390048 dosing and 2, 4, 8, 12, 16, 20, 24, 30, 36, 48, 60, and 72 h after MMV390048 dosing; then twice daily until parasitemia was not detected over a 48-h period; then 3-times per week until artemether-lumefantrine treatment; and at the end of the study (Day 28).

Sample size

The planned sample size of the first-in-human study (8 subjects per cohort) was calculated based on the assumption that if a dose of the study treatment is associated with a risk ≥50% for a toxicity-related event, then the probability that this event will be observed in at least one of the six subjects receiving MMV390048 is >98%. The planned sample size of the IBSM study (8 subjects per cohort) was calculated as previously described (21). Due to the exploratory nature of the formulation optimisation study, the planned sample size (9 subjects per cohort) was not based on formal statistical considerations but was deemed adequate to achieve the study objectives.

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CONFLICT OF INTEREST

SD, SC, CD, and JM are full time employees at Medicines for Malaria Venture (MMV), which sponsored and funded the three clinical trials reported here. HJ and GL are clinical research consultants contracted to MMV. UL is an employee of Richmond Pharmacology Limited and...
was the principal investigator of the formulation optimisation study. All other authors declare no competing interests.

**AUTHOR CONTRIBUTIONS**

KB (principal investigator), PS (lead investigator), HJ, KC, and EA were responsible for the acquisition of data in the first-in-human study and/or contributed to study design, analysis and interpretation of data. JMc was the principal investigator responsible for the acquisition of data in the IBSM study and contributed to study design, analysis and interpretation of data. UL was the principal investigator responsible for the acquisition of data in the formulation optimisation study and contributed to study design, analysis and interpretation of data. SC, SD, CD and JM are involved in the clinical development of MMV390048 and contributed to the study design of all three studies. LW developed the assay for measuring MMV390048 in plasma samples and performed the assays for all three studies. GL was the pharmacometrician involved in the study design and PK analyses for all three studies.

All authors have been involved in drafting the manuscript or revising it critically for important intellectual content and give final approval of the version to be published. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**DATA AVAILABILITY**

The data that support the findings of this study are available from Medicines for Malaria Venture upon reasonable request.
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### Table 1. Demographic and baseline characteristics by study and treatment group

|                | First-in-human study | IBSM study | Formulation optimisation study |
|----------------|----------------------|------------|-------------------------------|
|                | 5 mg (N=6)          | 20 mg (N=6) | 40 mg (N=6)          | 80 mg (N=6) | 120 mg (N=6) | 40 mg FED (N=6) | Placebo (N=12) | 20 mg (N=6) | 40 mg (N=9) | 40 mg (N=9) |
| Gender [n (%)] | Male                 | 6 (100)    | 4 (67)         | 5 (83)         | 6 (100)     | 4 (67)         | 11 (92)        | 6 (100)      | 9 (100)      | 9 (100)     |
|                | Female               | 0          | 2 (33)         | 2 (33)         | 1 (17)      | 0              | 2 (33)         | 1 (8)        | 0            | 0            |
| Race [n (%)]   | Black                | 4 (67)     | 4 (67)         | 4 (67)         | 6 (100)     | 4 (67)         | 9 (75)         | 0            | 1 (11)       | 1 (11)      |
|                | Mixed race           | 1 (17)     | 2 (33)         | 2 (33)         | 2 (33)      | 0              | 2 (33)         | 2 (17)       | 0            | 2 (22)      |
|                | Caucasian            | 0          | 0              | 0              | 0           | 0              | 1 (8)          | 6 (100)      | 5 (56)       | 7 (78)      |
|                | Other                | 1 (17)     | 0              | 0              | 0           | 0              | 0              | 0            | 1 (11)       | 1 (11)      |
| Age [years]    | Mean                 | 29.3       | 34.0           | 37.0           | 30.0        | 23.7           | 34.0           | 32.7         | 25.3         | 32.6        |
|                | SD                   | 11.0       | 10.0           | 7.6            | 12.1        | 4.7            | 10.7           | 11.7         | 4.0          | 10.4        |
| Height [cm]    | Mean                 | 171.8      | 167.3          | 171.5          | 168.7       | 169.8          | 170.7          | 170.5        | 182.3        | 182.4       |
|                | SD                   | 6.6        | 8.0            | 7.5            | 4.9         | 5.8            | 7.2            | 8.1          | 7.5          | 5.3         |
| Weight [kg]    | Mean                 | 69.1       | 69.8           | 72.4           | 67.5        | 66.4           | 73.6           | 65.8         | 81.4         | 85.9        |
|                | SD                   | 9.8        | 14.8           | 14.0           | 9.2         | 11.7           | 12.5           | 11.9         | 16.6         | 10.5        |
| BMI [kg/m²]    | Mean                 | 23.4       | 24.7           | 24.6           | 23.9        | 23.1           | 25.2           | 22.6         | 24.5         | 25.7        |
|                | SD                   | 3.2        | 3.2            | 4.1            | 4.4         | 4.6            | 3.5            | 3.0          | 4.8          | 2.2         |

IBSM: Induced blood stage malaria study; Form. A: Formulation A (tartaric acid tablets); Form. B: Formulation B (sylloid tablets); BMI: body mass index.
Table 2. Summary of adverse events by study and treatment group

| Number of subjects with adverse events (%) | First-in-human study | IBSM study | Formulation optimisation study |
|------------------------------------------|----------------------|------------|-------------------------------|
| All AEs                                  | 4 (67)               | 5 (83)     | 6 (100)                       |
| Treatment-related                        | 0                    | 3 (50)     | 2 (33)                        |
| Grade 2 AEs                              | 0                    | 4 (67)     | 5 (83)                        |
| Treatment-related                        | 0                    | 1 (17)     | 1 (17)                        |
| Grade 3 AEs                              | 0                    | 0          | 1 (17)                        |
| SAEs                                    | 0                    | 0          | 0                             |

Number of adverse events

| All AEs                                  | 11                   | 12           | 19                           |
| Treatment-related                        | 0                    | 3            | 3                            |
| Grade 2 AEs                              | 0                    | 5            | 4                            |
| Treatment-related                        | 0                    | 1            | 1                            |
| Grade 3 AEs                              | 0                    | 0            | 1                            |
| SAEs                                    | 0                    | 0            | 0                            |

583 IBSM: Induced blood stage malaria; Form. A: Formulation A (tartaric acid tablets); Form. B: Formulation B (sylloid tablets); AEs: adverse events; SAEs: serious adverse events. The Common Terminology Criteria for Adverse Events (CTCAE 4.03) was used to grade the severity of adverse events (grade 1-5). No adverse events of grade 4 or 5 severity were reported. * All grade 3 adverse events, and the serious adverse event, were considered to be possibly related to the study. + The grade 3 AEs recorded in the 40 mg and 40 mg FED cohort relate to the same subject. That is, a single subject experienced a grade 3 AE when enrolled in the 40 mg cohort and another grade 3 AE when enrolled in the 40 mg FED cohort. The AE was neutropenia in both instances. The grade 3 AE recorded in the placebo cohort was bullous dermatitis. The SAE recorded in the 120 mg cohort was generalised myoclonus.
Table 3. MMV390048 pharmacokinetic parameters by study and treatment group

| Dose       | C_{max} [ng mL^{-1}] | AUC_{0-inf} [ng h mL^{-1}] | CL/F [L h^{-1}] | Vz/F [L] | t_{1/2} [h] | t_{max} [h] |
|------------|-----------------------|-----------------------------|------------------|----------|-------------|-------------|
| **First-in-human study** |                       |                             |                  |          |             |             |
| 5 mg (N=6) | 15.6 (109.4)          | 2 144.0 (76.3)              | 2.3 (76.3)       | 519.8 (63.5) | 154.5 (51.0) | 1.0 (0.5-48.1) |
| 20 mg (N=6) | 136.2 (24.3)          | 33 274.0 (17.1)             | 0.6 (17.1)       | 265.5 (7.2)  | 306.1 (17.3) | 2.0 (1.0-3.0)  |
| 40 mg (N=6) | 74.4 (128.1)          | 17 594.9 (86.8)             | 2.3 (86.8)       | 624.2 (63.0) | 190.3 (64.7) | 2.5 (0.5-48.0) |
| 40 mg FED (N=6) | 154.7 (53.4)      | 34 494.7 (67.5)             | 1.2 (67.5)       | 373.4 (53.4) | 223.2 (48.3) | 4.0 (2.0-4.0)  |
| 80 mg (N=6) | 237.7 (110.3)         | 62 003.3 (46.0)             | 1.3 (46.0)       | 444.4 (64.0) | 238.7 (45.0) | 1.0 (1.0-96.0) |
| 120 mg (N=6) | 517.8 (85.5)          | 82 644.5 (165.8)            | 1.5 (165.8)      | 431.8 (101.1) | 206.1 (32.9) | 1.0 (1.0-3.0)  |
| **IBSM study** |                       |                             |                  |          |             |             |
| 20 mg (N=6) | 105.8 (42.4)          | 15 928.8 (43.7)             | NC               | NC       | NC          | 2.0 (1.0-3.0) |
| **Formulation optimisation study** |                       |                             |                  |          |             |             |
| Form. A 40 mg (N=9) | 271.0 (22.3) | 49 726.2 (53.5)             | 0.8 (53.5)       | 207.9 (20.7) | 179.1 (37.6) | 3.0 (1.0-4.0) |
| Form. B 40 mg (N=9) | 368.3 (18.0) | 57 608.2 (48.6)             | 0.7 (48.6)       | 149.4 (29.3) | 149.1 (44.0) | 3.0 (2.0-3.0) |

Data are geometric means (coefficient of variation) except for the t_{max}, which is median (range). IBSM: Induced blood stage malaria; Form. A: Formulation A (tartaric acid tablets); Form. B: Formulation B (syloid tablets); C_{max}: peak plasma concentration; AUC_{0-inf}: area under the concentration-time curve from 0 h to infinity; CL/F: apparent clearance; Vz/F: volume of distribution; t_{1/2}: elimination half-life; t_{max}: time at which C_{max} is reached; NC: Not calculated. *CL/F, Vz/F and t_{1/2} were not calculated for the IBSM study because the time interval over which MMV390048 was measured was not sufficient (less than two half-lives).
FIGURE LEGENDS

Figure 1. Subject flowchart for the first-in-human, induced blood stage malaria (IBSM) and formulation optimisation studies

Formulation A: tartaric acid tablets; Formulation B: syloid tablets.

Figure 2. MMV390048 plasma concentration time profiles by study and treatment group

Plots represent the geometric mean of each treatment group in the first-in-human study (A), IBSM study (B) and formulation optimisation study (C). IBSM: Induced blood stage malaria study; Formulation A: tartaric acid tablets; Formulation B: syloid tablets.

Figure 3. Time course of parasitemia in the induced blood stage malaria (IBSM) study

Subjects (N=6) were inoculated with ~1,800 viable *P. falciparum* parasites on Day 0 and a single dose of 20 mg MMV390048 was administered on Day 7 (indicated by the vertical dashed line). Artemether-lumefantrine (A/L) was administered to 4 subjects on Day 9 and to the remaining 2 subjects on Day 14 in response to recrudescence of asexual parasitemia. Plots represent the parasitemia for each subject. For the purpose of graphing the parasitemia data on a logarithmic scale, timepoints at which parasites could not be detected were substituted with a value of 1 parasite/mL.
First-in-human study

Subjects enrolled, N=40

5 mg, n=8

- MMV390048, n=6
- Placebo, n=2

20 mg, n=8

- MMV390048, n=6
- Placebo, n=2

40 mg, n=8

- MMV390048, n=6
- Placebo, n=2

80 mg, n=8

- MMV390048, n=6
- Placebo, n=2

120 mg, n=8

- MMV390048, n=6
- Placebo, n=2

IBSM study

Subjects enrolled, N=6

MMV390048, 20 mg, n=6

Formulation optimisation study

Subjects enrolled, N=18

MMV390048 tablet Formulation A (40 mg), n=9

MMV390048 tablet Formulation B (40 mg), n=9

40 mg FED, n=8
