CLINICAL STUDY PROTOCOL

A Phase 1b study to assess the antimalarial activity of MMV533 against *Plasmodium falciparum* 3D7 blood stage infection in healthy volunteers.

Protocol Number:	**MMV_MMV533_20_01**
Investigational Product:	**MMV533 for oral administration**
Principal Investigator:	**Dr Paul Griffin** **Q-Pharm Pty Ltd** **Level 5, 300C Herston Rd** **Herston Qld 4006, Australia** **Tel: +61 (0)7 3845 3636**
Local Sponsor/CRO:	**Southern Star Research Pty Ltd (SSR)** **1 Merriwa St** **Gordon, NSW 2072** **Australia** **Tel: +61 (0)2 9011 6266**
Global Sponsor:	**Medicines for Malaria Venture (MMV)** **ICC-Block G 3rd Floor** **20 route de Pré-Bois** **1215 Geneva 15** **Switzerland** **Tel: +41 22 555 03 00**
Sponsor Signatories:	**Dr Stephan Chalon, Medical Director** **Dr Amina Haouala, Clinical Science Lead**
Trial Medical Monitor:	**Dr Joanne Marjason** **Southern Star Research Pty Ltd** **Tel: +61 (0)427 959 156**
Date and Version:	**V1.0 01 March 2021**

1 PROTOCOL SUMMARY

1.1 PROTOCOL SYNOPSIS

Protocol Title	**A Phase 1b study to assess MMV533 antimalarial activity against *Plasmodium falciparum* 3D7 blood stage infection in healthy volunteers.**
IND Number	n/a
Protocol Number	MMV_MMV533_20_01
Local Sponsor	Southern Star Research Pty Ltd (SSR)
Global Sponsor	Medicines for Malaria Venture (MMV)
Principal Investigator	Dr Paul Griffin, Q-Pharm Pty Ltd
Phase of Development	Phase 1
Number of Study Sites	One Phase 1 clinical trial unit site will be conducting the study.
Treatment Groups	MMV533 (single, oral dose). 8 volunteers per cohort for 1-3 cohorts (8-24 volunteers total).
Volunteer Population	Healthy malaria-naïve adult volunteers
Investigational Medicinal Product (IMP)	MMV533, single dose taken orally (5 mg or 50 mg tablets) with 240 mL water under fasting conditions **Doses:** Starting dose: 20 mg as single oral dose in cohort 1A (4 participants). The MMV533 dose to be tested in the subsequent cohorts will be selected based on PK, PD and safety data from the preceding cohort and PK and safety data from the First-in-Human (FIH) study. Cohort 1B (4 volunteers) dose will be no more than 50 mg. Subsequent doses will not exceed a dose tested and deemed to be safe in the FIH study and will not exceed 200 mg MMV533.
Additional Study Treatment/Intervention: Malaria Challenge Agent **Non-IMP**	Malaria challenge agent for Induced Blood Stage Malaria (IBSM) model. Volunteers will be inoculated intravenously with a single dose of approximately 2,800 viable human erythrocytes infected with *Plasmodium falciparum* 3D7 in saline for injection. The total volume of the inoculum will be approximately 2 mL.
Additional Study Treatment: Malaria Rescue Medication **Non-IMP**	Mandatory Rescue Medication: • Artemether/lumefantrine (20 mg/120 mg; Riamet®). 6 oral doses of 4 tablets each at 0, 8, 24, 36, 48 and 60 hours (total 24 tablets), taken 240 mL full-fat milk. • Primaquine phosphate (equivalent 45 mg primaquine; Primacin™). One oral dose of 6 tablets (each with 13.2 mg primaquine phosphate, equivalent to 7.5 mg primaquine)

	taken with food; for volunteers with mild G6PD deficiency, 2 tablets taken orally as single dose with food (total dose 15 mg). Taken additionally to Riamet® or Malarone® at the discretion of the Investigator if required to ensure complete clearance of gametocytes. • Atovaquone/proguanil hydrochloride (250 mg/100 mg; Malarone®), only if volunteer has an intolerance to Riamet® (e.g. allergy), or contraindication to Riamet® develops or is suspected. Three-day course of 4 tablets taken once daily orally with food or milky drink. • Artesunate may be used if volunteers unable to tolerate oral antimalarial rescue treatment (eg, vomiting). Intravenous administration of 2.4 mg/kg at 0, 12, 24, 48 hours and then daily for up to 7 days or until able to take oral drugs.
Duration of Study per Volunteer	Screening 28 days prior to inoculation (Day -36 to Day -9), Inoculation and subsequent monitoring 8 days (Day -8 to Day -1), Confinement, Treatment and observation period 5 days (Day -1 to Day 5), follow-up including rescue treatment of 23 days until EOS (Day 5 to Day 28±3). Total study duration: up to 10 weeks from Screening until EOS.
Objectives	To assess in healthy adult volunteers: Primary: • To characterize the activity of single oral doses of MMV533 on clearance of *Plasmodium falciparum 3D7* blood stage parasites from the blood in an IBSM model Secondary: • To characterize the safety and tolerability of single oral doses of MMV533 administered after IBSM challenge • To characterize the pharmacokinetic parameters of single oral doses of MMV533 after IBSM challenge • To determine the relationship between MMV533 PK and asexual blood-stage parasitaemia in an IBSM model Exploratory: • To investigate drug resistance of *P. falciparum 3D7* after MMV533 administration
Endpoints	Primary endpoints: • Parasite reduction ratio over 48 hours (PRR_{48}) • Parasite clearance half-life ($Pt_{½}$) • Lag phase • Number of volunteers whose parasitaemia levels fall below limit of quantification (LOQ) following treatment with IMP • Number of volunteers with recrudescence of asexual parasitemia, defined as ≥5,000 blood stage parasites/mL after initial parasite clearance accompanied by either a two-fold parasitaemia increase within 48 hours, or re-

	occurrence of malaria symptoms with a malaria clinical score ≥6. • Time to recrudescence defined as the time at which asexual parasite levels reoccur after being below the LOQ or the time for which asexual parasitaemia levels are minimum, or, if not defined, the last observed time point without recrudescence. Secondary endpoints: **Safety** • Assessment of AEs/TEAEs; two phases will be defined: one corresponding to the inoculation phase (ie, 8 days before IMP administration), and one corresponding to treatment phase (i.e. 28 days after IMP administration) • Clinical laboratory evaluations including haematology, biochemistry, coagulation, urinalysis. • Vital signs (body temperature, blood pressure and heart rate supine), • 12-lead ECG (automatic reading): RR, HR, PR, QRS, QT, QTc measured by on site-device. **Pharmacokinetics of MMV533:** • Plasma parameters: at least C_{max}, t_{max}, AUC_{last}, AUC, $t_{½}$, CL/F, V_{ss}/F, Vz/F, plasma ratios of C_{max}, AUC_{last} and AUC **Pharmacokinetics-Pharmacodynamics:** • The PK/PD relationship between MMV533 plasma concentrations and blood stage asexual parasitaemia will be determined • Other key parameters will be derived from the PKPD model including minimum inhibitory concentration (MIC), the minimal parasiticidal concentration (MPC90), and the parasite reduction rate in 48 h (PRR48). **Exploratory endpoints**: • Perform *in vitro* drug sensitivity testing determining the 50% inhibitory concentrations (IC50) and the percentage surviving parasites
Study Description	The study will characterise the effects of single oral doses of MMV533 on the clearance of *Plasmodium falciparum 3D7* using the IBSM model. This is a well-established method for obtaining useful pharmacokinetic-pharmacodynamic (PK-PD) information on anti-malarial drugs. The study will be an open-label, pilot Volunteer Infection Study (VIS) using the IBSM model, single dose IMP, conducted in healthy malaria-naive adult volunteers infected with *Plasmodium falciparum 3D7*, up to 3 cohorts (n=8 per cohort receiving active

	treatment) enrolled in a sequential manner. Volunteers will be enrolled within a 28 day screening period to ensure volunteers meet all the inclusion criteria and none of the exclusion criteria. On Day -8 (8 days prior to IMP administration), volunteers will attend the clinical unit to be inoculated with the malaria challenge agent containing approximately 2,800 viable human erythrocytes infected with *P. falciparum 3D7* parasites. Parasitaemia will be monitored on an outpatient basis daily on Day -4, and then twice daily on Day -3, Day -2 and until admission to the clinical unit on Day -1 for eligibility check and confinement. Volunteers will be administered IMP on Day 1 and will have safety monitoring and blood sampling for parasitaemia monitoring and PK analysis on an inpatient basis for at least 108 hours post-IMP administration. Volunteers will be discharged from the clinical unit after review of ECG, vital signs, clinical and laboratory tests safety data by the Principal Investigator or delegate, and will then attend the clinical unit on an outpatient basis regularly for continued safety monitoring and blood sampling for parasitaemia monitoring and PK analysis. Volunteers will be administered mandatory malaria rescue medication at Day 21±3, or earlier if there is failure to clear parasites or recrudescence and at the discretion of the Principal Investigator or delegate. EOS will be on Day 28±3. Volunteers must have had at least two negative 18S qPCR results prior to EOS and must have completed their course of anti-malarial rescue medication. In the event of recrudescence, blood samples will be collected prior to rescue treatment to culture parasites for drug resistance testing.
Sample Size	Up to 24 volunteers inoculated, randomized and dosed (8 volunteers per group all receiving MMV533)
Inclusion Criteria	Volunteers must fulfil all the following inclusion criteria to be eligible to participate in the study: 1. Having given written informed consent prior to undertaking any study-related procedure. 2. Male or female aged between 18 to 55 years inclusive. 3. Available for the duration of the study and for 2 weeks following the End of Study Visit (EOS). 4. Lives with a spouse, family member, or housemate from the time of inoculation with the malaria challenge agent through to the EOS. 5. Total body weight greater than or equal to 50 kg, and a body mass index (BMI) within the range of 18 to 32 kg/m^2 (inclusive). 6. Willing to defer blood donations to a blood service for a minimum of 6 months after the EOS.

	7. Heterosexual **women of childbearing potential (WOCBP)** must agree to the use of a highly effective method of birth control (see below) combined with a barrier contraceptive from the screening visit until 30 days after the last dose of the IMP (covering a full menstrual cycle of 30 days starting after 5 half-lives of last dose of IMP) and have a negative result on urine pregnancy test performed before inoculation with the malaria challenge agent. *Note:* *a. Highly effective birth control methods include: combined (oestrogen and progestogen containing) oral/intravaginal/transdermal/implantable hormonal contraception associated with inhibition of ovulation, progestogen-only oral/injectable/implantable hormonal contraception associated with inhibition of ovulation, intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomised partner, or sexual abstinence or same sex relationship.* *b. Female volunteers who are abstinent (from penile-vaginal intercourse) must agree to start a double method if they start a sexual relationship with a male during the study. Female volunteers must not be planning in vitro fertilisation within the required contraception period.* 8. **Women of non-childbearing potential (WONCBP)** are defined as: *a. Natural (spontaneous) post-menopausal defined as being amenorrhoeic for at least 12 months without an alternative medical cause with a screening follicle stimulating hormone level (FSH) >25 IU/L (or at the local laboratory levels for post-menopause)* *b. Premenopausal with irreversible surgical sterilization by hysterectomy and/or bilateral oophorectomy or salpingectomy at least 6 months before screening (as determined by volunteer medical history)* 9. Males who have, or may have female sexual partners of child bearing potential during the course of the study must agree to use a double method of contraception including condom plus diaphragm, or condom plus intrauterine device, or condom plus stable oral/transdermal/injectable/implantable hormonal contraceptive by the female partner, from the time of informed consent through to 60 days (covering a spermatogenesis cycle) after the last dose of the IMP. Abstinent males must agree to start a double method if they begin sexual relationship with a female during the study and up to 60 days after the last dose of study drug. Males with female partners of child-bearing potential that are surgically sterile, or males who have undergone sterilisation and have had testing to confirm the success of the sterilisation, may

	also be included and will not be required to use above described methods of contraception. 10. Vital signs after 5 minutes resting in supine position: *a. Systolic blood pressure (SBP) - 90–140 mmHg,* *b. Diastolic blood pressure (DBP) - 40–90 mmHg,* *c. Heart rate (HR) 40–100 bpm.* 11. **At Screening and pre-inoculation** with the malaria challenge agent: normal standard mean of **triplicate 12-lead electrocardiogram (ECG)** parameters after 10 minutes resting in supine position in the following ranges: *a. QT ≤ 500 msec,* *b. QTcF ≤ 450 msec, QTcB ≤ 450 msec,* *c. PR interval ≤ 210 msec for both males and females, and* *d. Normal ECG tracing unless the Principal Investigator or delegate considers an ECG tracing abnormality to be not clinically relevant.* 12. In the opinion of the Principal Investigator or delegate, the individual has a high probability of adherence with and completion of the study, and willing and able to withdraw and refrain from restricted medications. 13. Certified as healthy by a comprehensive clinical assessment (detailed medical history and complete physical examination). 14. Fluent in English and able to understand and comply with written and verbal protocol-related requirements. 15. Agrees to adhere to the lifestyle considerations throughout the study (see Section 4.3.3) and is willing to consume 240 mL full-fat milk with each dose of rescue medication Riamet®.
Exclusion Criteria	If any of the following exclusion criteria apply, the potential volunteer will not be permitted to participate in the study: 1. Any lifetime history of malaria or participation in a previous malaria challenge study or malaria vaccine trial. 2. Must not have had malaria exposure that is considered significant by the Principal Investigator or delegate. This includes but is not limited to: - history of having travelled to or lived (> 2 weeks) in a malaria-endemic region during the past 12 months or planned travel to a malaria-endemic region during the course of the trial; - history of having lived for >1 year in a malaria-endemic region in the past 10 years; - history of having ever lived in a malaria-endemic region for more than 10 years inclusive. For endemic regions see https://malariaatlas.org/explorer/#/ Bali is not considered a malaria-endemic region.

	3. Presence of acute infectious disease and/or abnormal body temperature (defined as an a.m. tympanic temperature >37.5 ºC or a p.m. >37.7 ºC) at pre-inoculation.. 4. Haematology, biochemistry or urinalysis results that are outside of the laboratory normal reference ranges AND are either: - considered clinically significant by the Principal Investigator or delegate; OR - considered not clinically significant by the Principal Investigator or delegate BUT ARE ALSO outside of Sponsor-approved clinically acceptable laboratory ranges (Appendix 1) NOTE: Volunteers are not excluded if abnormal/out of laboratory normal reference range results are considered not clinically significant by the Principal Investigator or delegate AND are within the ranges specified in Appendix 1. 5. Breastfeeding or lactating; positive serum pregnancy test at screening, positive urine pregnancy test upon admission or at other timepoints as specified by schedule of activities tables. 6. Has previously received a blood transfusion 7. Use of antibiotics within 6 weeks of Screening. 8. Use of systemic therapies with antimalarial activity within 6 weeks of Screening. This includes (but not limited to) artemisinin, amodiaquine, atovaquone, chloroquine, mefloquine, mepacrine, primaquine, proguanil, quinine, sulfadoxine-pyrimethamine, benzodiazepine, flunarizine, fluoxetine, tetracycline, azithromycin, clindamycin, doxycycline, and tafenoquine. 9. Prior to screening and inoculation with the malaria challenge agent: • any systemic administration (oral, pulmonary/nasal, IV) of corticosteroids, anti-inflammatory drugs (excluding commonly used over-the-counter anti-inflammatory drugs such as ibuprofen, acetylsalicylic acid, diclofenac), immunomodulators or anticoagulants within the past three months. • Ibuprofen (preferred) may be used at doses of up to 1.2 g/day, or paracetamol at doses of up to 2 g/day after discussion with the Investigator. Limited use of other non-prescription medications or dietary supplements, not believed to affect subject safety or the overall results of the study, may be permitted on a case-by-case basis following approval by the Sponsor in consultation with the Investigator. • Any topical administration (cutaneous, eye drops) of corticosteroids within the past 2 weeks. • Any individual currently receiving or having previously received immunosuppressive therapy

	(including systemic steroids, adrenocorticotrophic hormone or inhaled steroids) at a dose or duration potentially associated with hypothalamic-pituitary-adrenal axis suppression within the past 12 months. 10. Any contra-indication to rescue medication according to the applicable labelling and if found to be severely G6PD deficient at screening (i.e. activity of less than 10% as per WHO definition). 11. Any history or presence of clinically relevant cardiovascular, pulmonary, gastrointestinal, hepatic/ gallbladder*/ bile duct, renal, metabolic, haematological, neurological, musculoskeletal, rheumatologic, systemic, ocular, gynaecologic (if female), infectious or autoimmune disease, or signs of acute illness. *including medical history of asymptomatic gallbladder stones. 12. History of recurrent headache (eg, tension-type, cluster or migraine) with a frequency of ≥2 episodes per month on average and severe enough to require medical therapy. History of recurrent nausea and/or vomiting (for vomiting only: more than twice a month). 13. Asthma (excluding childhood asthma, or mild asthma with preventative asthma medication required less than monthly and no event requiring treatment in the last 2 weeks prior to screening). 14. Any personal history of surgical procedures or disease that may affect IMP absorption, distribution (i.e. GI surgery, malabsorption sydromes) and metabolism or immune response to malaria inoculation (splenectomy). 15. Blood donation of any volume within one month before screening, or participation in any research study involving blood sampling (more than 450 mL/unit of blood). 16. Any documented evidence of current or past cardiovascular disease including: • cardiac arrhythmias or • family history of congenital long QT syndrome, Brugada syndrome, or unexplained sudden cardiac death. • Symptomatic postural hypotension at screening irrespective of the decrease in blood pressure, or asymptomatic postural hypotension defined as a decrease in systolic blood pressure ≥20 mmHg after 3 min of changing from supine to standing position. 17. Has evidence of increased cardiovascular risk (defined as >10%, 5-year risk for those greater than 35 years of age, as determined by the Australian Absolute Cardiovascular Disease Risk Calculator (http://www.cvdcheck.org.au). Risk factors include sex, age, systolic blood pressure (mm/Hg), smoking status, total and High-density lipoprotein (HDL) cholesterol (mmol/L) and reported diabetes status. *Note: The site investigator will perform cardiovascular risk calculation*

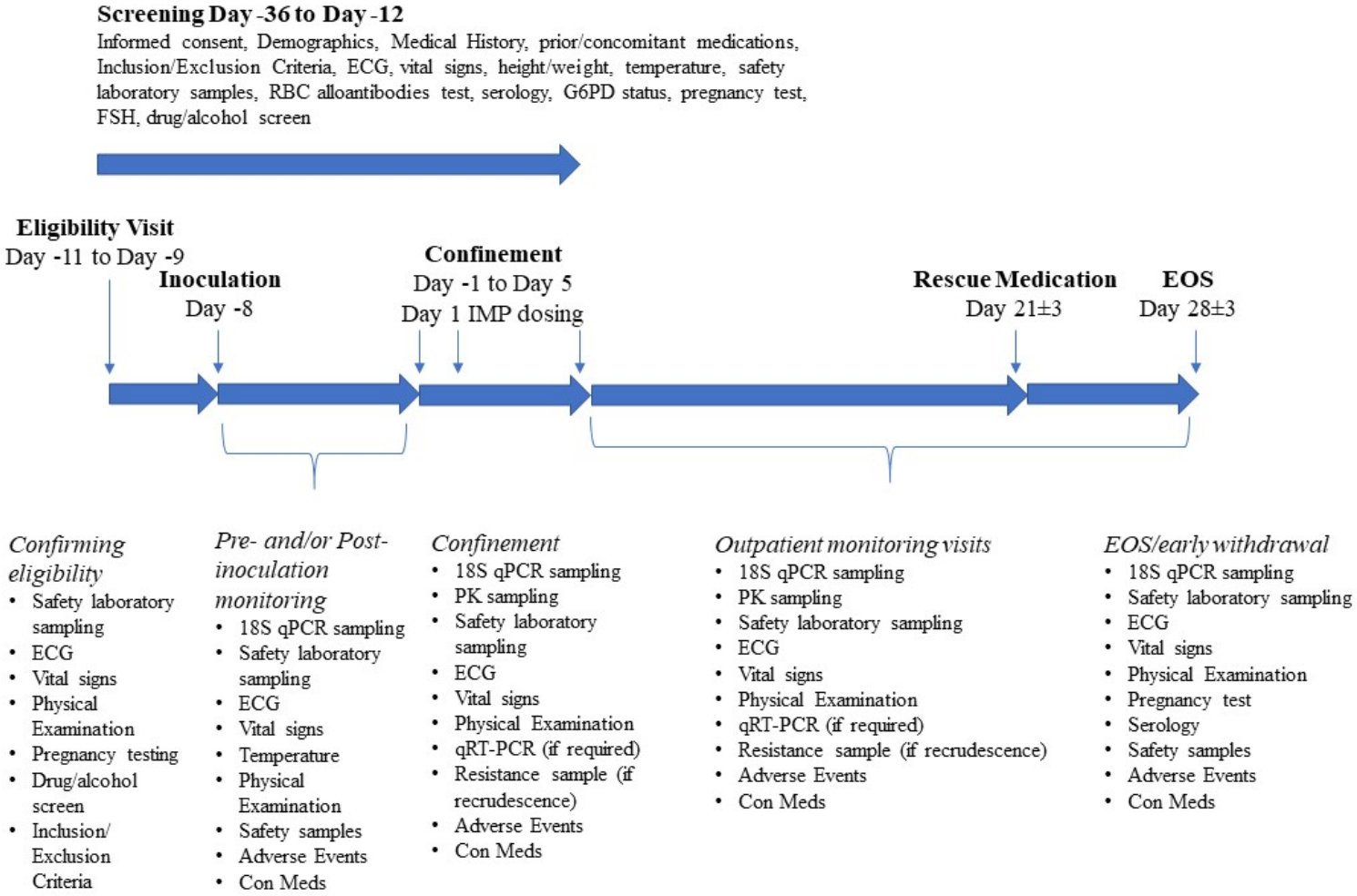
	once all assessments have been performed, prior to eligibility. 18. History or presence of diagnosed (by an allergist/immunologist) or treated (by a physician) food or known drug allergies, or any history of anaphylaxis or other severe allergic reactions including face, mouth, or throat swelling or any difficulty breathing. Individuals with known lactose or dairy intolerance are excluded. Volunteers with seasonal allergies/hay fever or allergy to animals or house dust mite that are untreated and asymptomatic at the time of dosing can be enrolled in the trial. 19. History of convulsion (including drug or vaccine-induced episodes). A medical history of a single febrile convulsion during childhood is not an exclusion criterion. 20. History of substance use disorder(s) within 5 years of screening and/or history of alcohol dependancy and/or any prior intravenous use of an illicit substance. 21. Smoked > 1 pack of cigarettes per day for > 10 years, or who currently (within 14 days prior to screening) smokes > 5 cigarettes per day. 22. Any individual who, in the judgement of the Principal Investigator or delegate, is likely to be noncompliant during the study, or unable to cooperate because of a language problem or poor mental development. 23. Any individual who cannot be contacted in case of emergency. 24. Any individual who is the Investigator, or delegates, research assistant, pharmacist, study coordinator, project manager, or other staff thereof, directly involved in conducting the study. 25. Any individual without a good peripheral venous access. 26. Participation in any investigational product study within the 12 weeks preceding inoculation with the malaria challenge agent or 5 times the half-life of the Investigational product, whichever is longer. 27. Positive serology test for hepatitis B (positive HB sAG or anti-HBc Ab), hepatitis C (anti-HCV) or human immune deficiency virus (HIV) (positive for anti-HIV1 and anti-HIV2 Ab). 28. Positive urine drug test at Screening, prior to inoculation with the malaria challenge agent or prior to IMP dosing. Any drug from the list of drugs tested (such as amphetamine, barbiturates, benzodiazepines, cocaine, methamphetamine, methadone, Opiates, phencyclidine, Tetrahydrocannabinol; and their metabolites) unless there is an acceptable explanation to the Principal Investigator or delegate (eg, volunteer has stated in advance that they consumed a prescription of over the counter product which contained the

	detected drug) and/or the volunteer has a negative urine drug screen on retest. 29. Positive alcohol screen at Screening, prior to inoculation with the malaria challenge agent or prior to IMP dosing. 30. Any consumption of citrus fruits (such as grapefruit, Seville oranges) or their juices within 7 days prior to IMP administration. 31. History of serious psychiatric condition that may affect participation in the study or preclude compliance with the protocol, including but not limited to: past or present psychoses, disorders requiring lithium, a history of attempted or planned suicide, more than one previous episode of major depression, any previous single episode of major depression lasting for or requiring treatment for more than 6 months, or any episode of major depression during the 5 years preceding screening. *Note: The Beck Depression Inventory will be used as an objective tool for the assessment of depression at screening. In addition to the conditions listed above, participants with a score of 20 or more on the Beck Depression Inventory and/or a response of 1, 2 or 3 for item 9 of this inventory (related to suicidal ideation) will not be eligible for participation. Participants with a Beck score of 17 to 19 may be enrolled at the discretion of the Investigator if they do not have a history of the psychiatric conditions mentioned in this criterion and their mental state is not considered to pose additional risk to the health of the volunteer or to the execution of the study and interpretation of the data gathered.* 32. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin or *in situ* cervical cancer considered treated and cured), treated or untreated, within 5 years of Screening, regardless of whether there is no evidence of local recurrence or metastases. 33. Any vaccination within 28 days of screening. 34. Any medical condition that in the opinion of the Principal Investigator or delegate would jeopardize the individual's involvement in the study.
Data Analysis	**Safety** Safety analysis (AE, laboratory parameters, vital signs, ECGs) will be based on the review of individual values, and descriptive statistics. The safety analysis will focus on the TEAE period, defined as the time from the first IMP administration up to and including EOS. AEs will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Their severity will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE V5.0 27 Nov 2017).

	The number (%) of volunteers experiencing TEAEs will be summarized by dose group. Safety analysis of ECGs will include in particular the number of volunteers with QTcF and/or QTcB prolongation of more than 30 ms and 60 ms and/or QTc > 450 msec during the study. **Pharmacodynamics** Individual total parasitemia (measured by 18S qPCR) and female gametocytemia (measured by *pfs25* RTPCR) profiles will be plotted. The individual asexual parasitemia profiles will be calculated as follows: if the gametocytemia level is lower than 10% of the total parasitemia level, the asexual parasitemia level is assumed to be equal to the total parasitemia level; otherwise, the asexual parasitemia is considered unknown. The asexual parasitemia profiles will be plotted individually and summarized by doses (median +/- 95% CI). The pharmacodynamic parameters (PCR, $Pt_{1/2}$, lag phase) calculated as described in Marquart et al[8] will be summarized using descriptive statistics (n, mean, geometric mean, standard deviation, median, Q1, Q3, minimum, and maximum) by dose group. The number and percentage of volunteers with absence of asexual parasitemia as well as the number and percentage of volunteers with recrudescence will be summarized by dose group. **Pharmacokinetics** MMV533 PK parameters will be calculated using non-compartmental methods from plasma concentration-time data and will be summarized by dose group using descriptive statistics. • Plasma PK parameters: at least C_{max}, t_{max}, AUC_{last}, AUC, $t_{½}$, CL/F, V_{ss}/F, Vz/F, plasma ratios of C_{max}, AUC_{last} and AUC **Pharmacokinetics/pharmacodynamics** • A population PKPD model will be derived from the plasma concentrations of MMV533 and asexual parasitemia levels in the challenge volunteers to quantify the relationship of plasma concentrations and the parasite killing/clearance. Several possible PD models may be tested and assessed by goodness-of-fit plots and BIC values to retain the best model. The estimated population PKPD parameters as well as their relative standard errors will be provided. Other key parameters will be derived from the PKPD model including minimum inhibitory concentration (MIC), the minimal parasiticidal concentration (MPC90), and the parasite reduction rate in 48 h (PRR48). **Exploratory parameters** If applicable, exploratory parameters will be summarized by treatment group using descriptive statistics and may be reported separately. Parasite drug resistance will be assessed by

	calculating the IC50 of IMP and determining the percentage of surviving parasites using *in vitro* drug sensitivity testing.

1.2 SCHEMA


Screening Day -36 to Day -12
Informed consent, Demographics, Medical History, prior/concomitant medications, Inclusion/Exclusion Criteria, ECG, vital signs, height/weight, temperature, safety laboratory samples, RBC alloantibodies test, serology, G6PD status, pregnancy test, FSH, drug/alcohol screen
Eligibility Visit
Day -11 to Day -9
Inoculation
Day -8
Confinement
Day -1 to Day 5
Day 1 IMP dosing
Rescue Medication
Day 21±3
EOS
Day 28±3
Confirming eligibility
• Safety laboratory sampling
• ECG
• Vital signs
• Physical Examination
• Pregnancy testing
• Drug/alcohol screen
• Inclusion/ Exclusion Criteria
Pre- and/or Post-inoculation monitoring
• 18S qPCR sampling
• Safety laboratory sampling
• ECG
• Vital signs
• Temperature
• Physical Examination
• Safety samples
• Adverse Events
• Con Meds
Confinement
• 18S qPCR sampling
• PK sampling
• Safety laboratory sampling
• ECG
• Vital signs
• Physical Examination
• qRT-PCR (if required)
• Resistance sample (if recrudescence)
• Adverse Events
• Con Meds
Outpatient monitoring visits
• 18S qPCR sampling
• PK sampling
• Safety laboratory sampling
• ECG
• Vital signs
• Physical Examination
• qRT-PCR (if required)
• Resistance sample (if recrudescence)
• Adverse Events
• Con Meds
EOS/early withdrawal
• 18S qPCR sampling
• Safety laboratory sampling
• ECG
• Vital signs
• Physical Examination
• Pregnancy test
• Serology
• Safety samples
• Adverse Events
• Con Meds

1.3 SCHEDULE OF ACTIVITIES

Table 1: Schedule of Activities Overview

	Screening	Pre-inoculation	Inoculation	Post-inoculation Phase				Treatment and Posttreatment phase														EOS/ ET
Day[a]	**D-36 to D-12**	**D-11 to D-9**	**D-8**	**D-4**	**D -3**	**D -2**	**D -1**	**D 1**	**D 2**	**D 3**	**D 4**	**D 5**	***D 6***	***D 7***	**D8 ± 2**	***D 10***	***D12***	**D14 ± 2**	***D18***	**D21 ± 3**	**D25 ± 3**	**D28 ± 3**
Informed consent	X																					
Confinement							X	X	X	X	X	X										
Discharge												X										
Outpatient visit at clinical site[a]	X	Xa	Xa	X	X	X							*X*a	*X*a	X	*X*a	*X*a	X	*X*a	X	X	X
Inclusion/exclusion criteria	X	X	X pre				X															
Medical/surgical history	X	X	X pre																			
BDI-II	X																					
Prior/concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study treatment administration																						
IV malaria challenge agent			Xb																			
MMV533 (IMP)								X														
Rescue medication																				Xa		
Safety																						
Physical exam - full	X																					X
Physical exam – symptom directed		X	X	X	X	X	X	X	X	X	X	X	*X*	*X*	X	*X*	*X*	X	*X*	X	X	
Height	X																					
Body weight	X	X																				X
G6PD status testing	X																					
Red blood cells antibodies	X																					X
Serology tests	X																					X
Urine drug screen, alcohol test[c]	X		X pre				X															
Vital signs supine and standing	X																					X
Vital signs supine			X pre & post	X	X	X	X	X	X	X	X	X	*X*	*X*	X	*X*	*X*	X	*X*	X pre	X	

	Screening	Pre-inoculation	Inoculation	Post-inoculation Phase				Treatment and Posttreatment phase													EOS/ ET
Day[a]	**D-36 to D-12**	**D-11 to D-9**	**D-8**	**D-4**	**D -3**	**D -2**	**D -1**	**D 1**	**D 2**	**D 3**	**D 4**	**D 5**	***D 6***	***D 7***	**D8 ± 2**	***D 10***	***D12***	**D14 ± 2**	***D18***	**D21 ± 3**	**D25 ± 3**	**D28 ± 3**
12-lead ECG	Xd		Xd pre				X	X	X	X	X	X			X			X		X pre	X	X
Body temperature[e]	X		X pre	X	X	X	X	X	X	X	X	X	*X*	*X*	X	*X*	*X*	X	*X*	X pre	X	X
Hematology, biochemistry, urinalysis	Xf	Xf			X		Xf	X	X	X	X	X			X			X		X pre	X	X
Coagulation	X																					
Pregnancy Test/ FSH[g]	X	X					X															X
Serum Safety samples[h]			Xh pre																			Xh
Diary card			X post	X	X	X	X						X	X	X	X	X	X		X pre	X	X
Malaria clinical score			X pre & post	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X pre	X	X
Adverse event collection[i]	Xi	Xi	Xi	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pharmacokinetics																						
MMV533 plasma samples								X	X	X	X	X			X			X		X		X
Pharmacodynamics																						
Parasitemia by qPCR			X pre	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
RT-PCR pfs25[j]								Xj														
Exploratory																						
Parasite drug resistance samples[k]													Xk									
OPTIONAL[l]																						
Research blood samples[l]		*Xl*						*Xl*							*Xl*							*Xl*

ABBREVIATIONS: BDI-II = Beck Depression Inventory; ECG = Electrocardiogram; EOS = End of study visit; ET = early termination visit; FSH = follicle stimulating hormone; IMP= Investigational Medicinal Product; NIMP= Non Investigational Medicinal Product; qPCR =quantitative polymerase chain reaction RT-PCR pfs25= gametocyte-specific mRNA transcript pfs25.

NOTE: See Table 2 and Table 3 for more detailed timepoint and procedural information from Day -11 (pre-inoculation) onwards. This table provides an overview only.

a. Volunteers will attend one visit between Days -11 to Day -9 to assess eligibility. One extra unscheduled visit is permitted if required for re-testing; results must be available prior to inoculation on Day -8. Eligibility must be confirmed prior to inoculation with the malaria challenge agent including from all assessments performed

pre-inoculation on Day -8. After inoculation with the malaria challenge agent on Day -8, clinical site staff will contact volunteers by telephone on Days -7 to Day -5 to check on well-being. Volunteers are expected to be asymptomatic; if AEs are reported within this period, volunteers will attend the clinical unit for assessment of vital signs, body temperature, 12-lead ECG, symptom-directed physical examination, malaria clinical scroe and possible blood sampling for 18S qPCR. Outpatient visits on Days 6, 7, 10, 12 and 18 are suggested days only and optional at the discretion of the Principal Investigator or delegate. As a guide, volunteers should return twice daily (morning and evening) until parasite counts are <~500 parasites/mL, when visits may then be once daily. When parasite counts are <~200 parasites/mL, visits may be on alternate days, and once parasite counts are negative or low and stable, visits may be reduced to 3 times per week until rescue medication is administered. Rescue medication may be administered prior to Day 21±3 as described in Section 5.5.2.

b. Volunteers will be inoculated with the malaria challenge agent and monitored for at least 60 minutes after inoculation with the malaria challenge agent before being permitted to leave the clinical unit. Volunteers will be issued with a volunteer card, diary and thermometer and educated on signs and symptoms of malaria. Post-inoculation, the Investigator will consider COVID-19 symptoms when assessing any reported symptoms as per Note in Section 6.1.4.

c. Drug test will be performed on urine sample. Alcohol test will be performed by breathalyser.

d. Triplicate 12-lead ECGs required at screening, Day -8 prior to inoculation with malaria challenge agent and Day 1 pre-dose. All other ECGs are 12-lead single ECGs.

e. Tympanic body temperature will be measured.

f. The results of safety laboratory testing (hematology, biochemistry and urinalysis) from Day -11 to Day -9 (pre-inoculation) and Day -1 (pre-IMP dosing) must be available for review by the Investigator prior to inoculation with malaria challenge agent on Day -8 and IMP dosing on Day 1 respectively. At least 8 hours fasting required prior to sampling at Screening, visit(s) between Day -11 to -9, and Day -1.

g. Serum β-human chorionic gonadotropin (hCG) pregnancy test for all female volunteers and FSH test for post-menopausal female volunteers at Screening. For WOCBP, urine β-hCG pregnancy test will be performed prior to inoculation with the malaria challenge agent on Day -8 and prior to IMP administration, and serum β-hCG pregnancy test conducted at EOS. If urine tests are positive, blood should be collected for serum β-hCG pregnancy test to confirm.

h. Safety blood samples (IMP safety serum sample and Inoculum safety serum sample) will be collected on Day -8 prior to inoculation with the malaria challenge agent and at EOS. Sample volume will be 5 mL per sample. See Section 7.6.

i. Untoward medical events recorded between screening and time of inoculation with malaria challenge agent will be recorded as medical history. Adverse events will be recorded continuously from time of inoculation with malaria challenge agent until EOS.

j. The exact day(s) of sampling will be at the discretion of the Investigator if gametocytaemia is suspected or confirmed.

k. One blood sample will be collected only if recrudescence occurs to investigate parasite drug resistance. This sample will be collected just prior to administration of rescue medication, and timing of sample collection will be flexible at the discretion of the Investigator in the event that rescue medication is administered early due to recrudescence. Recrudescence is defined as ≥5000 blood stage parasites per mL after initial parasite clearance accompanied by either a two-fold parasitaemia increase within 48 hours, or reoccurrence of malaria symptoms with a malaria clinical score of ≥6.

l. OPTIONAL: These research samples may only be collected at the timepoints indicated if the volunteer has consented to the separate QIMR study under the local Sponsorship of QIMR Berghofer Institute – see Section 7.6. Sample on Day 1 is pre-dose.

Table 2: Day -11 to Day 1 Detailed Schedule of Activities

Day -11 to Day 1	**D-11 to D-9**	**D -8a**	**D-4**	**D-3**		**D-2**		**D-1**			**D1**											
Time hour (H) minutes[b]				**am**	**pm**	**am**	**pm**	**am**	**pm**	**Pre-dose**	**0H**	**0H 30**	**1H**	**1H 30**	**2H**	**3H**	**4H**	**6H**	**8H**	**10H**	**12H**	**16H**	**20H**
Confinement								X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Outpatient visit at clinical site	X	Xc	X	X	X	X	X	X															
Inclusion/exclusion criteria	X	Xc						X															
Medical/surgical history	X	Xc																					
Concomitant medications	X	Xc	X	X	X	X	X	X															
Inclusion		Xc																					
Study treatment administration																							
IV malaria inoculum (NIMP)		Xd																					
MMV533 (IMP)											Xe												
Rescue medication (NIMP)[f]											Xf												
Safety[g]																							
Physical examination		Xc, h	Xh	Xh	Xh	Xh	Xh	Xh	Xh	Xh		Xh											
Body weight	X																						
Urine drug screen, alcohol test[i]		Xc						X															
Vital signs (supine)[j]		Xj	X	X	Xk	X	X	X	X	X			X		X		X	X	X	X	X		
12-lead ECG[l]		Xc,l						X		Xl							X	X	X		X		
Body temperature		Xc	X	X	Xk	X	X	X	X	X			X		X		X	X	X	X	X		

Day -11 to Day 1	**D-11 to D-9**	**D -8a**	**D-4**	**D-3**		**D-2**		**D-1**			**D1**											
Time hour (H) minutes[b]				**am**	**pm**	**am**	**pm**	**am**	**pm**	**Pre-dose**	**0H**	**0H 30**	**1H**	**1H 30**	**2H**	**3H**	**4H**	**6H**	**8H**	**10H**	**12H**	**16H**	**20H**
Haematology, biochemistry, urinalysis	Xm			Xm LFT				Xn		X													
Urine β-hCG (WOCBP only)	Xo							Xo															
Serum safety samples		Xc																					
Diary card		Xp	X	X	Xk	X	X	X															
Malaria clinical score		Xj	X	X	Xk	X	X	X	X	X					X			X			X		
Adverse event collection[q]	Xq	X	X	X	X	X	X	X															
Pharmacokinetics																							
MMV533 + metabolites plasma samples										X		X	X	X	X	X	X	X	X	X	X		
Pharmacodynamics																							
Parasitaemia by qPCR		Xc	X	Xr	Xr	Xr	Xk, r	Xr	Xk, r	X					X		X	X	X		X	X	X
OPTIONAL[s]																							
Research blood samples[s]	*Xs*									*Xs*													

ABBREVIATIONS: ECG = electrocardiogram; IMP = investigational medicinal product; NIMP = noninvestigational medicinal product; qPCR =quantitative polymerase chain reaction; qRT-PCR pfs25= Reverse-transcription polymerase chain reaction for the gametocyte-specific mRNA transcript pfs25; WOCBP = woman of child bearing potential.

a. After inoculation with the malaria challenge agent on Day -8, clinical site staff will contact volunteers by telephone daily on Days -7 to Day -5 to check on well-being. There are no outpatient visits to the clinical site scheduled for Days -7 to -5 as volunteers are expected to be asymptomatic. If AEs are reported during this period, volunteers will attend the clinical unit for assessment of vital signs, body temperature, 12-lead ECG, symptom-directed physical examination, malaria clinical score and

possible blood sampling for 18S qPCR at discretion of the Investigator. Post-inoculation, the Investigator will consider COVID-19 symptoms when assessing any reported symptoms as per Note in Section 6.1.4.

b. Time (hour/minute) is expressed in reference to the single administration of IMP at t = 0 (T0H).
c. Activities/procedures to be conducted prior to inoculation with the malaria challenge agent.
d. Eligible volunteers will be injected with malaria challenge agent containing approximately 2800 viable human erythrocytes infected with *P. falciparum* 3D7 paraiste in 2 mL saline and then monitored for at least 60 minutes before being permitted to leave the clinical unit.All volunteers attending on the same day will be inoculated within a 60 minute period and within 4 hours of preparation of the malaria challenge agent.
e. A fasting single oral dose of MMV533 (IMP) will be administered with 240 mL water. Dose will depend on cohort. Post-IMP: volunteers to remain seated for 10 minutes, stay semi-recumbent for next 2 hours (except if safety assessments require lying position), no food for 4 hours and no liquids for 1 hour. Prior to IMP dosing on Day 1, the Principal Investigator or delegate should ensure that no contraindication to administration of IMP are present as described in Section 4.3.8.1 (in which case rescue medication should be administered instead of IMP).
f. Rescue medication may be given earlier than Day 21±3 or instead of IMP on Day 1 in the event of: withdrawal of inoculated volunteer and/or as outlined in Section 4.3.8.1 and Section 5.5.2.
g. Refer to Safety Section 7 for detailed safety investigations.
h. Symptom-directed physical examination only. Body systems will be reviewed only if clinically indicated and at the discretion of the Principal Investigator or delegate.
i. Drug test will be performed on urine sample. Alcohol test will be performed by breathalyzer.
j. Vital signs (supine for 5 minutes; heart rate beats per minute; blood pressure diastolic and systolic mmHg). Vital signs and malaria clinical score will be performed before and approximately 60 minutes after inoculation at Day -8.
k. At discretion of the Principal Investigator or delegate.
l. Automatic reading. Triplicate standard 12-lead ECGs required prior to inoculation and on Day 1 pre-IMP dose. Single standard 12-lead ECGs at all other timepoints.
m. Participants will have fasted for at least 8 hours prior to sampling at visit/s between Day -11 to Day -9. Results of clinical safety laboratory tests conducted at the visit/s during Day -11 to -9 must be available for review prior to inoculation on Day -8. Liver funtction tests (LFTs) are the only safety laboratory tests required on Day -3.
n. Participants will have fasted for at least 8 hours prior to sampling on Day -1. The results of clinical safety laboratory tests conducted on Day -1 must be available and reviewed by the Principal Investigator or delegate prior to administration of IMP on Day 1.
o. If urine tests are positive, blood should be collected for serum β-hCG pregnancy test to confirm.
p. After inoculation with the malaria challenge agent and prior to leaving the clinical unit, each volunteer will be issued a volunteer card, diary and thermometer to record temperature in the event of fever, and to record adverse events and concomitant medications. Volunteers will be educated on the signs and symptoms of malaria and how to fill in the diary. Volunteers should bring diaries to each subsequent visit to the clinical unit.
q. Untoward medical events prior to time of inoculation with malaria challenge agent will be recorded as medical history. Adverse events will be recorded from time of inoculation with malaria challenge agent until EOS.
r. Samples for 18s qPCR will be collected twice daily or at the discretion of the Principal Investigator or delegate.
s. OPTIONAL: These research samples may only be collected at the timepoints indicated if the volunteer has consented to the separate QIMR study under the local Sponsorship of QIMR Berghofer Institute – see Section 7.6.

NOTE:

- When several assessments take place at the same time, the following order should be respected: ECG, vital signs, blood sampling, drug administration, meal.

- To respect exact timing of pharmacokinetic samples, the other measures will be done ahead of the scheduled time.
- Diary cards and a thermometer will be issued to volunteers for completion at home (including temperature monitoring and recording AEs and concomitant medications). Volunteers will be educated on the signs and symptoms of malaria and how to complete the diary prior to leaving the clinical unit.
- Time windows for PK and PD sampling are described in Table 5.
- Time windows for assessments conducted from Day 1 are described per assessment section (if applicable) in Section 7.

Table 3: Day 2 to Day 28 Detailed Schedule of Activities

Study Day	**D2**			**D3**		**D4**		**D5**		***D6***		***D7***	**D8 ±2**	***D10***	***D12***	**D14 ±2**	***D18***	**D21 ±3**		**D25 ±3**	**EOS D28 ±3**
Time (hours post-IMP)[a]	**24 H**	**30 H**	**36 H**	**48 H**	**60 H**	**72 H**	**84 H**	**96H**	**108 H**	**120 H**	**132 H**	**144 H**	**168 H**	**192 H**	**264 H**	**312H**	**408 H**	**Pre**	**480H**	**576H**	**648H**
Confinement	X	X	X	X	X	X	X	X	X												
Discharge									X												
Outpatient visit at clinical site										*Xb*	*Xb*	*Xb*	X	*Xb*	*Xb*	X	*Xb*	X	X	X	X
Concomitant medications	X									*Xb*	*Xb*	*Xb*	X	*Xb*	*Xb*	X	*Xb*	X	X	X	X
Study treatment administration																					
Rescue medication (Non-IMP)[c]																			X[c]		
Safety[d]																					
Physical examination	Xe			Xe		Xe		Xe	Xf	*Xe,b*	*Xb*	*Xe, b*	Xe	*Xeb*	*Xe,b*	Xe	*Xe,b*	Xe	Xe	Xe	Xe
Body weight																					X
Urine drug screen, alcohol test[g]																					
Vital signs[h]	X	X	X	X	X	X	X	X	X	*Xb*	*Xb*	*Xb*	X	*Xb*	*Xb*	X	*Xb*	X		X	Xh
12-lead ECG[i]	X			X		X		X					X			X		X		X	X
Body temperature	X	X	X	X	X	X	X	X	X	*Xb*	*Xb*	*Xb*	X	*Xb*	*Xb*	X	*Xb*	X		X	X
Serology																					X
Red blood cells antibodies																					X
Hematology, biochemistry,	X			X		X		X					X			X		X		X	X
Urinalysis	X					X							X			X		X			X
Serum β-hCG (WOCBP only)																					X

Study Day	**D2**			**D3**		**D4**		**D5**		***D6***		***D7***	**D8 ±2**	***D10***	***D12***	**D14 ±2**	***D18***	**D21 ±3**		**D25 ±3**	**EOS D28 ±3**
Time (hours post-IMP)[a]	**24 H**	**30 H**	**36 H**	**48 H**	**60 H**	**72 H**	**84 H**	**96H**	**108 H**	**120 H**	**132 H**	**144 H**	**168 H**	**192 H**	**264 H**	**312H**	**408 H**	**Pre**	**480H**	**576H**	**648H**
Serum safety samples[j]																					Xj
Diary card review										*Xb*	*Xb*	*Xb*	X	*Xb*	*Xb*	X	*Xb*	X		X	X
Malaria clinical score	X	X	X	X	X	X	X	X	X	*Xb*	*Xb*	*Xb*	X	*Xb*	*Xb*	X	*Xb*	X		X	X
Adverse event collection	X									*Xb*	*Xb*	*Xb*	X	*Xb*	*Xb*	X	*Xb*	X	X	X	X
Pharmacokinetics																					
MMV533 plasma PK samples	X			X		X		X					X			X		X			X
Pharmacodynamics																					
Parasitaemia by 18S qPCR	X	X	X	X	X	X	X	Xk	Xk	*Xbk*	*Xbk*	*Xb*	X	*Xb*	*Xb*	X	*Xb*	X		X	X
RT-PCR pfs25								Xl													
Exploratory																					
Parasite drug resistance samples[m]										Xm								Xm			
OPTIONAL[n]																					
Research blood samples[n]													*Xn*								*Xn*

ABBREVIATIONS: ECG = electrocardiogram; IMP = investigational medicinal product; NIMP = noninvestigational medicinal product; qPCR =quantitative polymerase chain reaction; qRT-PCR pfs25= Reverse-transcription polymerase chain reaction for the gametocyte-specific mRNA transcript pfs25; WOCBP = woman of child bearing potential.

a. Time (hours) is expressed in reference to the single administration of IMP at t = 0 hours (H).
b. Procedures/activities will be conducted at the discretion of the Principal Investigator or delegate Visits to the clinical unit on Days 6, 7, 10, 12 and 18 are suggested days only and optional at the discretion of the Principal Investigator or delegate. Adverse events will continue to be recorded at each outpatient visit and if reported or observed at other times. As a guide, volunteers should return twice daily (morning and evening) until parasite counts are <~500 parasites/mL, when visits may then be once daily. When parasite counts are <~200 parasites/mL, visits may be on alternate days, and once parasite counts are negative or low and stable, visits may be reduced to 3 times per week until rescue medication is administered.

c. Volunteers will be administered mandatory malaria rescue medication (Riamet®) on Day 21±3, or earlier if there is failure of clearance or recrudescence of parasitemia or at the discretion of the Principal Investigator or delegate or due to volunteer withdrawal or safety issue (see Section 4.3.8.1 and Section 5.5.2). In case of early rescue treatment administration, associated safety assessments must be continued as per the Schedule of Activities for rescue medication. Malaria clinical score and parasitemia by qPCR should be continued for up to 72h as per the Schedule of Activities and must be performed as a minimum 72h post rescue treatment administration or at the discretion of the Principal Investigator or delegate. Malarone® may be given if allergy or contraindication to Riamet® occurs or is suspected. The first dose of oral antimalarial rescue treatment will be administered at the clinical unit, with subsequent doses taken by the volunteer at home and recorded in the diary. Clinical unit staff must contact the volunteer on a daily basis during the dosing period to confirm compliance. Intravenous Artesunate may be given if volunteer unable to tolerate oral antimalarial rescue treatment. Primacin™ will be administered at the discretion of the Investigator in addition to Riamet® if gametocytaemia is indicated. Post-inoculation, the Investigator will consider COVID-19 symptoms when assessing any reported symptoms as per Note in Section 6.1.4.
d. Refer to Safety Section 7 for detailed safety investigations,
e. Full physical examination at EOS only. Symptom-directed physical examination at all other designated timepoints - body systems to be reviewed only if clinically indicated and at the discretion of the Principal Investigator or delegate.
f. Prior to volunteer discharge from confinement, clinical unit nursing staff must confirm with the Principal Investigator or delegate if any volunteer requires a symptom-driven examination before discharge.
g. Unscheduled drug and alcohol screening may be performed if required for volunteer safety or to assess continued eligibility at the discretion of the Principal Investigator or delegate. Drug test will be performed on urine sample. Alcohol test will be performed by breathalyzer.
h. Vital signs (supine for 5 minutes; heart rate beats per minute; blood pressure diastolic and systolic mmHg;).Supine and standing vital signs (measured after supine 5 minutes then after standing for 3 minutes) will be performed at EOS.
i. Automatic reading. Single standard 12-lead ECGs.
j. Safety blood samples (IMP safety serum sample and Inoculum safety serum sample) will be collected on Day -8 prior to inoculation with the malaria challenge agent and at EOS. Sample volume will be 5 mL per sample. See Section 7.6.
k. Samples for 18s qPCR will be collected at the discretion of the Principal Investigator – see footnote (b). Two negative 18s qPCR results are required prior to EOS.
l. Samples for qRT-PCR will be collected from Day 5 onwards at the discretion of the Principal Investigator and if 18 qPCR data suggest presence of gametocytes in volunteers' blood.
m. Blood samples will be collected prior to first dose of rescue medication only if recrudescence occurs to investigate parasite drug resistance. Recrudescence is defined as ≥ 5000 blood stage parasites per mL after initial parasite clearance accompanied by either a two-fold parasitaemia increase within 48 hours, or reoccurrence of malaria symptoms with a malaria clinical score ≥6.
n. OPTIONAL: These research samples may only be collected at the timepoints indicated if the volunteer has consented to the separate QIMR study under the local Sponsorship of QIMR Berghofer Institute – see Section 7.6.

NOTE:

- When several items take place at the same time, the following order should be respected: ECG, vital signs, blood sampling, drug administration, meal.
  To respect exact timing of pharmacokinetic samples, the other measures will be done ahead of the scheduled time.

- The diary card issued to the volunteer on D-8 will be checked at each outpatient visit. Volunteers will enter into the diary cards data relating to their daily physical activity, daily alcohol consumption, any symptoms of malaria and if applicable, their oral temperature. Volunteers will bring their completed diary cards with them to the trial site for all ambulant, Follow-up, and EOS visits.
- Days 6, 7, 10, 12 and 18 are optional at the discretion of the Principal Investigator or delegate – see footnote (b).

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ACT	Artemisinin-based combination therapy
ADME	Absorption, distribution, metabolism, excretion
AE	Adverse event
Ae0-t	Cumulated amount of drug excreted in urine from time 0 to time t
ALT	Alanine aminotransferase
AST	Aspartate transaminase
AUC	Area under the curve
BDI	Beck Depression Inventory
BMI	Body mass index
CLR0-t	Renal clearance of drug estimated from time 0 to time t
C_{max}	Maximum plasma concentration observed
DBP	Diastolic blood pressure
DME/T	Drug metabolism enzymes and drug transporters
ECG	Electrocardiogram
EOS	End of study visit
FDA	Food and Drug Administration (US)
fe0-t	Fraction of dose of drug excreted in urine from time 0 to time t
FSH	Follicle stimulating hormone
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HR	Heart rate
HREC	Human Research Ethics Committee
IBSM	Induced blood stage malaria
IC_{50}	Concentration for 50% inhibition
IMP	Investigational medicinal product
LFT	Liver function tests
LOQ	Limit of quantification
MCB	Master cell bank
MMV	Medicines for Malaria Venture
MPC	Minimum drug concentration above which parasite clearance is maximal
OTC	Over the counter
PBPK	Physiologically based pharmacokinetics
PD	Pharmacodynamics
PK	Pharmacokinetics
PKPD	Pharmacokinetic-pharmacodynamic
PRR_{48}	Parasite reduction ratio over 48 hours

$Pt_{1/2}$	Parasite clearance half-life
SAD	Single ascending dose
SBP	Systolic blood pressure
SCID	Severe combined immunodeficiency (mouse model)
SRC	Safety Review Committee
SSR	Southern Star Research Pty Ltd
$t_{1/2z}$	Terminal half-life
TEAE	Treatment emergent adverse event
t_{lag}	Lag time
t_{max}	Time to reach maximum plasma concentration (C_{max})
VIS	Volunteer infection study
Vss/F	Apparent volume of distribution at the steady state after single dose
Vz/F	Apparent volume of distribution after single dose
WHO	World Health Organisation

PROTOCOL APPROVAL/ SIGNATURES

I herewith approve the following protocol entitled "**A Phase 1b study to assess the safety, tolerability and antimalarial activity of MMV533 against *Plasmodium falciparum* blood stage infection in healthy volunteers.**", Version 1.0. dated 01 March 2021. Effective date 02 March 2021.

SPONSOR SIGNATURE

Signature:	Stephan Chalon
Name:	Dr Stephan Chalon MD PhD
Role:	Medical Director, Medicines for Malaria Venture
Date:	01/ 03 /2021

Signature:	
Name:	Dr Amina Haouala PhD
Role:	Clinical Science Lead, Medicines for Malaria Venture
Date:	01 / 03 /2021

PRINCIPAL INVESTIGATOR SIGNATURE

A Phase 1b study to assess the safety, tolerability and antimalarial activity of MMV533 against *Plasmodium falciparum* blood stage infection in healthy volunteers.

MMV_MMV533_20_01

Version: 1.0, 01 March 2021

Issue Date: 02 March 2021

Principal Investigator Agreement

I have read the above-mentioned protocol and am aware of my responsibilities as Principal Investigator for this study. As such, I agree to:

- Personally supervise the conduct of this trial;
- Conduct the trial in accordance with International Conference on Harmonization (ICH) E6 Good Clinical Practice: Consolidated Guidance (GCP), applicable regulatory requirements, and the protocol;
- Comply with the procedures for data recording and reporting as required by the regulatory authorities and the Sponsor;
- Permit monitoring, auditing, and inspection of study records as required by ICH GCP;
- Retain the essential clinical study documents as required by ICH GCP and the Sponsor.

Principal Investigator Signature:	Electronically signed by: Paul Griffin Reason: I have approved this document. Date: Sep 14, 2021 10:51 GMT+10
Principal Investigator Name:	Paul Griffin
Date:	14-Sep-2021 / 2021

2 INTRODUCTION AND RATIONALE

MMV533 is in development as a potential single dose cure, fast acting and long-lasting treatment for malaria. At the writing of this study protocol, the very First In Human study with MMV533 has been initiated in Australia in August 2020.

2.1 DISEASE BACKGROUND

Malaria continues to be a challenge globally. In 2018, some 228 million cases of malaria occurred worldwide, resulting in an estimated 405,000 deaths.[1] Although the incidence rate of malaria globally is declining, the rate of decline has slowed dramatically, remaining at similar levels to that reported in 2014. In 2018, nineteen countries in sub-Saharan Africa and India accounted for almost 85% of global malaria cases, and 94% of all malaria deaths were from the African region.

Malaria is a cyclical parasitic infection of humans and female *Anopheles* mosquitoes.[2] As a brief summary, after a female *Anopheles* mosquito feeds on the blood of a human infected with the malarial parasite it is asymptomatically infected with the parasite. The parasites grow and multiply in the gut of the mosquito before migrating to its salivary glands, and the infected mosquito then injects some of the infected saliva into the next human it feeds on. In the human, the parasites first migrate to the liver before infecting red blood cells, where the cycle continues with successive generations of asexual parasites invading and destroying red blood cells. These blood stage parasites cause the symptoms of malaria. After a period of time, some of the blood stage parasites differentiate into male and female forms (gametocytes) which continue the cycle of infection when ingested by a female *Anopheles* mosquito feeding on the infected human. Worldwide, *Plasmodium falciparum* is the predominant malaria parasite, causing the vast majority of cases in Africa and many cases in South-East Asia, Eastern Mediterranean region and the Western Pacific Region.[1]

The World Health Organisation (WHO) promotes several strategies to help combat malaria, including preventing mosquito bites with use of insecticide-treated mosquito nets while sleeping and insecticide spraying of indoor walls, use of preventative therapies especially for vulnerable groups such as children and pregnant women, and improve improving institutional structures for accessing care to diagnose and treat malaria.[1] For treatment of malaria, artemisinin-based combination therapy (ACT) has been widely implemented, with an estimated 3 billion courses of ACT procured globally between 2010 and 2018. However, parasite resistance to artemisinin and other therapies are becoming more common especially in Thailand and other South-East Asian countries, while mosquito resistance to insecticide is also widespread.

Therefore, there remains an urgent need to continue the clinical development of novel, effective and well-tolerated anti-malarial drugs, of which MMV533 is a promising candidate.

2.2 INVESTIGATIONAL MEDICINAL PRODUCT - MMV533

MMV533 is a first in class, fast acting, orally bioavailable blood stage inhibitor of *Plasmodium falciparum* with a long half-life. Its mechanism of action is unknown. MMV533 is being developed for the curative treatment of uncomplicated malaria caused by *P. falciparum* in adults and children, and has been evaluated in a range of preclinical pharmacological models of malaria.

2.2.1 Clinical Studies

MMV533 is being tested in humans in the ongoing First-in-Human (FIH) study MMV_MMV533_19_01 investigating safety, tolerability and pharmacokinetics of MMV533 in healthy adult male and female volunteers.

At the time of writing of this document, the first two cohorts (5 and 10 mg) of the MMV533 FIH study had completed (n=16 volunteers; 4 placebo and 12 IMP). No serious or severe AEs were observed, and Cohort 3 (20 mg) commenced in January 2021.

Preliminary human PK analysis obtained from the ongoing FIH (study MMV_MMV533_19_01), show a long half-life of approximately 120 hours and acceptable intersubject variability. Observed peak concentrations and $AUC_{0\text{-}inf}$ at 5 and 10 mg MMV533 (Table 4) showed higher than anticipated exposure. This therefore suggests that the highest dose to be evaluated in the FIH will not exceed 200 mg MMV533 in order not to exceed the AUC cap (125 µg.h/ml) identified for this project.

Table 4: Observed (5 and 10 mg MMV533) and Predicted (20-400 mg MMV533) mean group AUC inf for each MMV533 dose level evaluated in the FIH Study

Dose MMV533 (mg)	AUC_{inf} (µg/mL*hr)	Safety Margin - Dog NOAEL (125 µg/mL*hr)
5	3.0	41.2
10	6.1	20.6
20	12.1	10.3
50	30.3	4.1
100	60.7	2.1
200	121.3	1.0
400	242.7	0.5

ABBREVIATIONS: AUC = area under the curve; FIH = first-in-human; NOAEL = no observed adverse effect level; PK = pharmacokinetics.

2.2.2 Non-Clinical Studies

MMV533 has previously been evaluated extensively by Sanofi in nonclinical studies as compound SAR441121. SAR441121 was acquired by MMV and renamed as 'MMV533' (short for MMV688533). Refer to the Investigator's Brochure for further details.

MMV533 has been shown to act on malarial parasite Ring/Schizont erythrocytic stages, and has high potency *in vitro* with an IC_{50} of 4-8 nM on a panel of sensitive and resistant strains of *Plasmodium falciparum*, 2.5-21 nM on a panel of field and mutant strains and 0.8-18 nM on field isolates.

MMV533 has been shown to be highly efficacious with a single oral dose against *Plasmodium falciparum in vivo* in a severe combined immunodeficiency (SCID) mouse model. MMV533 also exhibited an outstanding resistance profile after no mutant selection was observed after 6 months of drug pressure.

As observed in animals, a high bioavailability of MMV533 is expected in human based on the good *in vitro* permeability (~ $30.10^{-7}cm^{-1}$ in standard condition). No major food effect is anticipated in human with this compound. *In vitro* investigations in human hepatocytes in primary culture showed MMV533 is very slowly metabolized. Based on this experiment, predicted *in vivo* metabolic clearance values of MMV533 correspond to 0.6, 1.4 and 1.0% of human hepatic blood flow at 0.1, 1 and 10 µM, respectively. Complementary investigation with a model allowing longer incubation time (HepatoPac) suggest involvement of CYP3A4 and possibly CYP2D6 at low concentration (0.1µM) in the metabolic clearance. MMV533 showed *in vitro* competitive inhibition of CYP2B6, 2C8, 2C9, 2C19, 2D6 with apparent Ki values of 12.6, 0.783, 2.39, 8.99 and 4.04 µM, respectively and exhibited time-dependent inhibition of CYP3A with KI and kinact values of 28 µM and 0.036 min-1, respectively. Moreover, there is a risk of increase in exposure of drug sensitive substrates of these CYP isoforms when co-administered with MMV533. This compound was not identified as an inducer of CYP1A1/2, CYP2B6, or CYP3A4. Based on allometric scaling and physiologically based pharmacokinetic (PBPK) modelling, the predicted $t_{1/2z}$ in humans was approximately 100 hours.

MMV533 was found to be a P-glycoprotein (P-gP) inhibitor on Caco-2 cell line with digoxin as PgP substrate (IC_{50}:108 µM). As such, it cannot be ruled out that co-administration of MMV533 will affect the pharmacokinetics of a sensitive substrate such as digoxin.

An IC50 of 85.6µM was obtained for MMV533 following vitro bile salt export pump (BSEP) inhibition assay. This result suggests that the interaction of MMV533 with BSEP at the clinical level is unlikely.

2.2.3 Safety and Risk/Benefit Assessment

At the time of writing of this protocol, single dose administration of MMV533 is being evaluated in the FIH study MMV_MMV533_19_01. Two dose levels, 5 and 10 mg MMV533, have been fully evaluated in cohorts of 8 healthy volunteers (6 active drug/2 placebo). The data, which are currently blinded and preliminary, did not show serious or severe AEs nor any clinically concerning events from ECGs, vital signs and safety laboratory tests. As shown in Table 4, the 10 mg dose was associated with a group mean $AUC_{0\text{-}inf}$, which is 21-fold below the AUC cap identified for the FIH study.

The risks to the volunteers in this study will be managed by frequent safety monitoring, including close monitoring during periods of confinement at the clinical unit when a single oral dose of MMV533 will be administered as the investigational medicinal product (IMP). Safety monitoring will include clinical laboratory safety tests (chemistry, haematology, serology, urinalysis), physical examination, vital signs and frequent ECG analysis. Throughout the study, the safety and PK data will be assessed by the Principal Investigator, Sponsor Medical Director and Sponsor Medical Monitor prior to commencing the study participation of the next planned cohort.

See also Section 4.4 for risk management of other study treatments/interventions (non-IMP).

2.3 RATIONALE

2.3.1 Rationale for the Study

MMV533 is being developed for the curative treatment of uncomplicated malaria caused by *P. falciparum* in adults and children as a single dose regimen in a fixed-dose combination with another non-artemisinin antimalarial drug.

One way to expedite the evaluation of novel anti-malarial drugs is to use volunteer infection studies (VIS) such as the induced blood stage malaria (IBSM) model.[3,4] In the IBSM model, healthy volunteers are inoculated with an established master cell bank (MCB) of donor red blood cells infected with *P. falciparum 3D7*. The subsequent parasitaemia can be monitored closely and clinically to evaluate the effect of the IMP on malaria infection. The IBSM is an attractive tool to test the activity of drugs in nonimmune subjects in a rapid and cost-effective manner. The antimalarial activity of a treatment can be determined by analysing parasitaemia clearance in subjects after drug treatment by quantitative polymerase chain reaction (qPCR).[5,6,7]

This study is an IBSM Phase1b study of MMV533 that will be performed in healthy adult volunteers with no history of malaria to exclude bias related to previous malarial infection, underlying disease or pathological conditions on the safety and PK parameters.

The study will characterise the effects of single oral doses of MMV533 on the clearance of *Plasmodium falciparum* using the IBSM model in healthy adult volunteers. This study will be open-label, and is a well-established method for obtaining useful pharmacokinetic-pharmacodynamic (PKPD) information on anti-malarial drugs.

2.3.2 Rationale for the Dose

A human dose that could maintain blood concentrations above MPC for 100 hours (corresponding to 2 parasite erythrocyte cycles) was identified as a target minimal efficacious dose for the treatment of acute uncomplicated *Plasmodium falciparum* malaria. Using observed preliminary human PK data from the FIH study (5 and 10 mg cohorts) and pharmacodynamic parameters estimated from preclinical studies, the predicted efficacious human dose of MMV533 is estimated to be 20 to 35 mg to achieve 9 log parasite clearance and 25 to 40 mg to achieve 12 log parasite clearance .

The two doses of MMV533 (single-dose) administered to volunteers enrolled in Cohort 1 (N=8) of the IBSM model study will be determined based on the safety, tolerability, and PK results obtained in the FIH study MMV_MMV533_19_01 and taking into account the human efficacious dose predicted from preclinical studies.

Cohort 1 will be split into two sequential sub-cohorts of 4 participants each:

- Cohort 1A: the first four participants will receive a single oral dose of MMV533 of 20 mg.
- Cohort 1B: the highest dose of MMV533 to be tested in the 4 remaining participants enrolled in Cohort 1 will not exceed a dose already tested and deemed to be safe in the FIH study, and will not exceed 50 mg (i.e. Cohort 4 of the FIH).

There will be no safety review in-between cohorts 1A and 1B.

Cohorts 2 and 3 will be composed of up to 4 dose groups with a minimum of 2 volunteers per dose group. If more than one dose level are tested in a given cohort, volunteers will be randomised after inoculation day but prior to Day 8 and administered a single oral dose of MMV533. The doses administered to Cohort 2 and Cohort 3 (maximum of 8 dosed volunteers per cohort) will be selected based on PK, PD and safety data from the preceding cohort and PK and safety data from the FIH study. Prior to inoculation of the next cohort, the Safety Review Committee (SRC) will review safety and tolerability data up to Day 22 and PK and PD analysis outcomes based on PD and PK data up to Day 15 from the previous cohort. The maximum dose used in Cohort 2 and 3 will not exceed a dose tested and deemed to be safe in the FIH study and will not exceed 200 mg.

3 STUDY OBJECTIVES AND ENDPOINTS

PRIMARY OBJECTIVES	PRIMARY ENDPOINTS
• To characterize the activity of single oral doses of MMV533 on clearance of *Plasmodium falciparum 3D7* blood stage parasites from the blood in an IBSM model	• Parasite reduction ratio over 48 hours (PRR_{48})[8] • Parasite clearance half-life ($Pt_{½}$)[8] • Lag phase[8] • Number of volunteers whose parasitemia levels fall below the limit of quantification (LOQ) following treatment with IMP • Number of volunteers with recrudescence, , defined as ≥5,000 blood stage parasites/mL after initial parasite clearance accompanied by either a two-fold parasitaemia increase within 48 hours, or re-occurrence of malaria symptoms with a malaria clinical score ≥6. • Time to recrudescence defined as the time at which asexual parasite levels reoccur after being below the LOQ or the time for which asexual parasitaemia levels are minimum, or, if not defined, the last observed time point without recrudescence
SECONDARY OBJECTIVES	**SECONDARY ENDPOINTS**
• To characterize the safety and tolerability of single oral doses of MMV533 administered after IBSM challenge	• Assessment of AEs/TEAEs; two phases will be defined: one corresponding to the inoculation phase (ie, 8 days before IMP administration), and one corresponding to treatment phase (i.e., 28 days after IMP administration) • Clinical laboratory evaluations including haematology, biochemistry, coagulation, urinalysis. • Vital signs (body temperature, blood pressure and heart rate supine), • 12-lead ECG (automatic reading): RR, HR, PR, QRS, QT, QTc measured by on site-device.

SECONDARY OBJECTIVES	SECONDARY ENDPOINTS
• To characterize the pharmacokinetic (PK) parameters of single oral doses of MMV533 after IBSM challenge	• Plasma parameters: at least C_{max}, t_{max}, AUC_{last}, AUC, $t_{½}$, CL/F, V_{ss}/F, Vz/F, plasma ratios of C_{max}, AUC_{last} and AUC
• To determine the relationship between MMV533 PK and asexual blood-stage parasitaemia in an IBSM model	• The Pharmacokinetics-Pharmacodynamics (PK/PD) relationship between MMV533 plasma concentrations and blood stage asexual parasitaemia will be determined • Other key parameters will be derived from the PKPD model including the minimum inhibitory concentration (MIC), the minimal parasiticidal concentration (MPC90), and the parasite reduction rate in 48 h (PRR48).

EXPLORATORY OBJECTIVES	EXPLORATORY ENDPOINTS
• To investigate drug resistance of *P. falciparum 3D7* after MMV533 administration	• Perform *in vitro* drug sensitivity testing determining the 50% inhibitory concentrations (IC50) and the percentage surviving parasites

4 INVESTIGATIONAL PLAN

4.1 DESCRIPTION OF OVERALL STUDY DESIGN AND PLAN

The study is a Phase 1b, open label, adaptive study using the Induced Blood Stage Malaria (IBSM) model to characterise the antimalarial activity of a single oral dose of the IMP MMV533 using pharmacokinetic and pharmacodynamic parameters and to further assess the safety and tolerability of MMV533.

4.1.1 Induced Blood Stage Malaria (IBSM)

The study is an open label, volunteer infection study (VIS), single dose study in healthy malaria-naïve adult volunteers. Up to 3 cohorts of 8 volunteers each will be enrolled in a sequential manner, as has been conducted in previous trials and published.[3,4,9,10] The study will be initiated before the FIH study MMV_MMV533_19_01 is complete, with each dose of MMV533 tested in this study being first considered safe in the FIH study. Cohort 1 of this study will test a maximal dose of 50 mg MMV533, while cohort 2 and 3 will evaluate doses not exceeding 200 mg if this dose is considered safe and tolerable in the FIH study.

The method used for this VIS is the Induced Blood Stage Malaria (IBSM) model, which is an established model conducted previously at the clinical site. In the IBSM model, volunteers are inoculated with a malaria challenge agent as described in Sections 5.2 and 5.7.2.

Study assessments and procedures are as scheduled in Section 1.3 and as described in Sections 6 and 7. Briefly, potential volunteers will be screened between Day -36 and Day -12, with written informed consent obtained prior to any study procedures and eligibility to be assessed according to the inclusion and exclusion criteria. Eligibility will be confirmed at the clinical visit between Days -11 and Day -8, with the results of clinical safety laboratory tests reviewed at the visit on Day -8 prior to inoculation with the malaria challenge agent. Volunteers will be inoculated with the malaria challenge agent on Day -8 and monitored for a period of at least 60 minutes afterwards. All volunteers attending on the same day will be inoculated within a 60 minute period and within 4 hours of preparation of the inoculum. Clinical unit staff will contact volunteers daily on Days -7 to -5 to check on well-being and AEs/conmeds. Volunteers may attend the clinical unit for an unscheduled safety visit(s) during this period at the discretion of the Principal Investigator or delegate.

Volunteers will attend outpatient visits at the clinical unit on Days -4 to -1 for blood sampling for malaria 18S qPCR and safety assessments. Volunteers will be confined to the clinical unit from Day -1, and administered a single oral dose of IMP as per Section 5.1 on Day 1. Volunteers will be confined for at least 108 hours after IMP dosing for safety monitoring, and blood sampling for malaria 18S qPCR and PK analysis. Volunteers may be discharged from the clinical unit from 108 hours post-IMP administration if considered clinically well by the Principal Investigator or delegate.

A blood sample for malaria 18S qPCR will be collected at approximately 108 hours post MMV533 treatment. Volunteers may then return morning and evening until parasite counts are <~500 parasites/mL or at the Investigator's discretion. Volunteers may then have daily visits at the Investigator's discretion. When parasite counts are <~200 parasites/mL, sampling may revert to alternate day visits at the Investigator's discretion. Once the malaria 18S qPCR results are negative, or low and stable, volunteers may be

reviewed 3 times per week at the Investigator's discretion until mandatory rescue medication (Riamet®) treatment.

Post-confinement monitoring will be based on parasitaemia results rather than set days, particularly if more than 1 dose of MMV533 is administered within a cohort. Visit days in Table 3 except for Days 8±2, 14±2, 21±3, 25±3 and EOS are provided as a guide only.

Outpatient visits will be for continued safety monitoring and blood sampling for PK and malaria 18S qPCR. From Day 5, blood samples may also be taken for qRT-PCR to test for presence of gametocytes.

An approved regimen for malaria treatment will be administered as rescue medication to all volunteers beginning on Day 21 (±3 days). Volunteers will be administered Riamet®, and also Primacin™ at the discretion of the Investigator if gametocytes are indicated or suspected. If an intolerance or contraindication to Riamet® develops or is suspected, Malarone® will be administered as rescue medication. Artesunate (IV) may be administered if a volunteer is unable to tolerate oral antimalarial treatment with Riamet® or Malarone®.

Rescue treatment may be administered earlier at the discretion of the Investigator, including if there is a failure of clearance of parasites or recrudescence. Recrudescence is defined as ≥ 5000 blood stage parasites/mL accompanied by either a 2-fold increase within 48 hours as measured by malaria 18S qPCR, or reoccurrence of malaria symptoms with a malaria clinical score ≥6. Blood samples will be collected during recrudescence to culture parasites *in vitro* for drug resistance testing.

Volunteers will attend the end of study visit (EOS) on Day 28 (±3).

4.2 DISCUSSION OF STUDY DESIGN INCLUDING CHOICE OF CONTROL GROUPS

The study design is a well-established VIS model, and the clinical site is experienced in using the IBSM model to evaluate potential anti-malarial drugs.

The study is open label to assess the safety and PK of IMP after malaria infection respectively. Control groups are not required or appropriate.

4.3 SELECTION OF STUDY POPULATION

The intended patient population for MMV533 is people infected with *Plasmodium falciparum*, but this study will be performed in healthy adult volunteers with no history of malaria to exclude any bias related to previous *Plasmodium falciparum* infection, underlying disease or pathological conditions, on the safety and PK parameters.

4.3.1 Inclusion Criteria

Volunteers must fulfil all the following inclusion criteria to be eligible to participate in the study:

1. Having given written informed consent prior to undertaking any study-related procedure.
2. Male or female aged between 18 to 55 years inclusive.
3. Available for the duration of the study and for 2 weeks following the End of Study Visit (EOS).
4. Lives with a spouse, family member, or housemate from the time of inoculation with the malaria challenge agent through to the EOS.

5. Total body weight greater than or equal to 50 kg, and a body mass index (BMI) within the range of 18 to 32 kg/m^2 (inclusive).
6. Willing to defer blood donations to a blood service for a minimum of 6 months after the EOS.
7. Heterosexual women of childbearing potential (WOCBP) must agree to the use of a highly effective method of birth control (see below) combined with a barrier contraceptive from the screening visit until 30 days after the last dose of the IMP (covering a full menstrual cycle of 30 days starting after 5 half-lives of last dose of IMP) and have a negative result on urine pregnancy test performed before inoculation with the malaria challenge agent.

   Note:

   a. *Highly effective birth control methods include: combined (oestrogen and progestogen containing) oral/intravaginal/transdermal/implantable hormonal contraception associated with inhibition of ovulation, progestogen-only oral/injectable/implantable hormonal contraception associated with inhibition of ovulation, intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomised partner, or sexual abstinence or same sex relationship.*

   b. *Female volunteers who are abstinent (from penile-vaginal intercourse) must agree to start a double method if they start a sexual relationship with a male during the study. Female volunteers must not be planning in vitro fertilisation within the required contraception period.*

8. Women of non-childbearing potential (WONCBP) are defined as:

   a. *Natural (spontaneous) post-menopausal defined as being amenorrhoeic for at least 12 months without an alternative medical cause with a screening follicle stimulating hormone level (FSH) >25 IU/L (or at the local laboratory levels for post-menopause)*
   b. *Premenopausal with irreversible surgical sterilization by hysterectomy and/or bilateral oophorectomy or salpingectomy at least 6 months before screening (as determined by volunteer medical history)*

9. Males who have, or may have female sexual partners of child bearing potential during the course of the study must agree to use a double method of contraception including condom plus diaphragm, or condom plus intrauterine device, or condom plus stable oral/transdermal/injectable/implantable hormonal contraceptive by the female partner, from the time of informed consent through to 60 days (covering a spermatogenesis cycle) after the last dose of the IMP. Abstinent males must agree to start a double method if they begin sexual relationship with a female during the study and up to 60 days after the last dose of study drug. Males with female partners of child-bearing potential that are surgically sterile, or males who have undergone sterilisation and have had testing to confirm the success of the sterilisation, may also be included and will not be required to use above described methods of contraception.
10. Vital signs after 5 minutes resting in supine position:

    a. *Systolic blood pressure (SBP) - 90–140 mmHg,*
    b. *Diastolic blood pressure (DBP) - 40–90 mmHg,*
    c. *Heart rate (HR) 40–100 bpm.*

11. At Screening and pre-inoculation with the malaria challenge agent: normal standard mean of triplicate 12-lead electrocardiogram (ECG) parameters after 10 minutes resting in supine position in the following ranges:
    a. *QT ≤ 500 msec,*
    b. *QTcF ≤ 450 msec, QTcB ≤ 450 msec*
    c. *PR interval ≤ 210 msec for both males and females, and*
    d. *Normal ECG tracing unless the Principal Investigator or delegate considers an ECG tracing abnormality to be not clinically relevant.*
12. In the opinion of the Principal Investigator or delegate, the individual has a high probability of adherence with and completion of the study, and willing and able to withdraw and refrain from restricted medications.
13. Certified as healthy by a comprehensive clinical assessment (detailed medical history and complete physical examination).
14. Fluent in English and able to understand and comply with written and verbal protocol-related requirements.
15. Agrees to adhere to the lifestyle considerations throughout the study (see Section 4.3.3) and is willing to consume 240 mL full-fat milk with each dose of rescue medication Riamet®.

4.3.2 Exclusion Criteria

If any of the following exclusion criteria apply, the potential volunteer will not be permitted to participate in the study:

1. Any lifetime history of malaria or participation in a previous malaria challenge study or malaria vaccine trial.
2. Must not have had malaria exposure that is considered significant by the Principal Investigator or delegate. This includes but is not limited to:
    - history of having travelled to or lived (> 2 weeks) in a malaria-endemic region during the past 12 months or planned travel to a malaria-endemic region during the course of the trial;
    - history of having lived for >1 year in a malaria-endemic region in the past 10 years;
    - history of having ever lived in a malaria-endemic region for more than 10 years inclusive. For endemic regions see https://malariaatlas.org/explorer/#/ Bali is not considered a malaria-endemic region.
3. Presence of acute infectious disease and/or abnormal body temperature (defined as an a.m. tympanic temperature >37.5 ºC or a p.m. >37.7 ºC) at pre-inoculation.
4. Haematology, biochemistry or urinalysis results that are outside of the laboratory normal reference ranges AND are either:
    - considered clinically significant by the Principal Investigator or delegate; OR
    - considered not clinically significant by the Principal Investigator or delegate BUT ARE ALSO outside of Sponsor-approved clinically acceptable laboratory ranges (Appendix 1: Sponsor Approved Clinically Acceptable Inclusion Laboratory Ranges)

    NOTE: Volunteers are not excluded if abnormal/out of laboratory normal reference range results are considered not clinically significant by the Principal Investigator or

delegate AND are within the ranges specified in Appendix 1: Sponsor Approved Clinically Acceptable Inclusion Laboratory Ranges.

5. Breastfeeding or lactating; positive serum pregnancy test at screening, positive urine pregnancy test upon admission or at other timepoints as specified by schedule of activities tables.
6. Has previously received a blood transfusion.
7. Use of antibiotics within 6 weeks of Screening.
8. Use of systemic therapies with antimalarial activity within 6 weeks of Screening. This includes (but not limited to) artemisinin, amodiaquine, atovaquone, chloroquine, mefloquine, mepacrine, primaquine, proguanil, quinine, sulfadoxine-pyrimethamine, benzodiazepine, flunarizine, fluoxetine, tetracycline, azithromycin, clindamycin, doxycycline, and tafenoquine.
9. Prior to screening and inoculation with the malaria challenge agent:

- any systemic administration (oral, pulmonary/nasal, IV) of corticosteroids, anti-inflammatory drugs (excluding commonly used over-the-counter anti-inflammatory drugs such as ibuprofen, acetylsalicylic acid, diclofenac), immunomodulators or anticoagulants within the past three months.
- Ibuprofen (preferred) may be used at doses of up to 1.2 g/day, or paracetamol at doses of up to 2 g/day after discussion with the Investigator. Limited use of other non-prescription medications or dietary supplements, not believed to affect subject safety or the overall results of the study, may be permitted on a case-by-case basis following approval by the Sponsor in consultation with the Investigator.
- Any topical administration (cutaneous, eye drops) of corticosteroids within the past 2 weeks.
- Any individual currently receiving or having previously received immunosuppressive therapy (including systemic steroids, adrenocorticotrophic hormone or inhaled steroids) at a dose or duration potentially associated with hypothalamic-pituitary-adrenal axis suppression within the past 12 months.

10. Any contra-indication to rescue medication according to the applicable labelling and if found to be severely G6PD deficient at screening (i.e. activity of less than 10% as per WHO definition).
11. Any history or presence of clinically relevant cardiovascular, pulmonary, gastrointestinal, hepatic/ gallbladder*/ bile duct, renal, metabolic, haematological, neurological, musculoskeletal, rheumatologic, systemic, ocular, gynaecologic (if female), infectious or autoimmune disease, or signs of acute illness. *including medical history of asymptomatic gallbladder stones.
12. History of recurrent headache (eg, tension-type, cluster or migraine) with a frequency of ≥2 episodes per month on average and severe enough to require medical therapy. History of recurrent nausea and/or vomiting (for vomiting only: more than twice a month).
13. Asthma (excluding childhood asthma, or mild asthma with preventative asthma medication required less than monthly and no event requiring treatment in the last 2 weeks prior to screening).

14. Any personal history of surgical procedures that may affect IMP absorption, distribution (i.e. GI surgery) and metabolism or immune response to malaria inoculation (splenectomy).
15. Blood donation of any volume within one month before screening, or participation in any research study involving blood sampling (more than 450 mL/unit of blood).
16. Any documented evidence of current or past cardiovascular disease including:
    - cardiac arrhythmias or
    - family history of congenital long QT syndrome, Brugada syndrome, or unexplained sudden cardiac death.
    - Symptomatic postural hypotension at screening irrespective of the decrease in blood pressure, or asymptomatic postural hypotension defined as a decrease in systolic blood pressure ≥20 mmHg within 3 min when changing from supine to standing position.
17. Has evidence of increased cardiovascular risk (defined as >10%, 5-year risk for those greater than 35 years of age, as determined by the Australian Absolute Cardiovascular Disease Risk Calculator (http://www.cvdcheck.org.au). Risk factors include sex, age, systolic blood pressure (mm/Hg), smoking status, total and High-density lipoprotein (HDL) cholesterol (mmol/L) and reported diabetes status. *Note: The site investigator will perform cardiovascular risk calculation once all assessments have been performed, prior to eligibility.*
18. History or presence of diagnosed (by an allergist/immunologist) or treated (by a physician) food or known drug allergies, or any history of anaphylaxis or other severe allergic reactions including face, mouth, or throat swelling or any difficulty breathing. Individuals with known lactose or dairy intolerance are excluded. Volunteers with seasonal allergies/hay fever or allergy to animals or house dust mite that are untreated and asymptomatic at the time of dosing can be enrolled in the trial.
19. History of convulsion (including drug or vaccine-induced episodes). A medical history of a single febrile convulsion during childhood is not an exclusion criterion.
20. History of substance use disorder(s) within 5 years of screening and/or history of alcohol dependancy and/or any prior intravenous use of an illicit substance.
21. Smoked > 1 pack of cigarettes per day for > 10 years, or who currently (within 14 days prior to screening) smokes > 5 cigarettes per day.
22. Any individual who, in the judgement of the Principal Investigator or delegate, is likely to be noncompliant during the study, or unable to cooperate because of a language problem or poor mental development.
23. Any individual who cannot be contacted in case of emergency.
24. Any individual who is the Investigator, or delegates, research assistant, pharmacist, study coordinator, project manager, or other staff thereof, directly involved in conducting the study.
25. Any individual without a good peripheral venous access.
26. Participation in any investigational product study within the 12 weeks preceding inoculation with the malaria challenge agent or 5 times the half-life of the Investigational product, whichever is longer.

27. Positive serology test for hepatitis B (positive HB sAG or anti-HBc Ab), hepatitis C (anti-HCV) or human immune deficiency virus (HIV) (positive for anti-HIV1 and anti-HIV2 Ab).
28. Positive urine drug test at Screening, prior to inoculation with the malaria challenge agent or prior to IMP dosing. Any drug from the list of drugs tested (such as amphetamine, barbiturates, benzodiazepines, cocaine, methamphetamine, methadone, Opiates, phencyclidine, Tetrahydrocannabinol; and their metabolites) unless there is an acceptable explanation to the Principal Investigator or delegate (eg, volunteer has stated in advance that they consumed a prescription of over the counter product which contained the detected drug) and/or the volunteer has a negative urine drug screen on retest.
29. Positive alcohol screen at Screening, prior to inoculation with the malaria challenge agent or prior to IMP dosing.
30. Any consumption of citrus fruits (such as grapefruit, Seville oranges) or their juices within 7 days prior to IMP administration.
31. History of serious psychiatric condition that may affect participation in the study or preclude compliance with the protocol, including but not limited to: past or present psychoses, disorders requiring lithium, a history of attempted or planned suicide, more than one previous episode of major depression, any previous single episode of major depression lasting for or requiring treatment for more than 6 months, or any episode of major depression during the 5 years preceding screening.

    Note: The Beck Depression Inventory will be used as an objective tool for the assessment of depression at screening. In addition to the conditions listed above, participants with a score of 20 or more on the Beck Depression Inventory and/or a response of 1, 2 or 3 for item 9 of this inventory (related to suicidal ideation) will not be eligible for participation. Participants with a Beck score of 17 to 19 may be enrolled at the discretion of the Investigator if they do not have a history of the psychiatric conditions mentioned in this criterion and their mental state is not considered to pose additional risk to the health of the volunteer or to the execution of the study and interpretation of the data gathered.
32. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin or *in situ* cervical cancer considered treated and cured), treated or untreated, within 5 years of Screening, regardless of whether there is no evidence of local recurrence or metastases.
33. Any vaccination within 28 days of screening.
34. Any medical condition that in the opinion of the Principal Investigator or delegate would jeopardize the individual's involvement in the study.

4.3.3 Lifestyle Considerations

Volunteers will be requested to follow a stable lifestyle while participating in the study, with no increased physical activity compared to their usual habits. The volunteers will also be requested to adhere to the following restrictions, and should be reminded of them throughout the study:

- Not to consume citrus fruits such as grapefruit or Seville oranges or their juices, particularly 7 days prior to IMP administration on Day 1;

- Not to consume food or beverages containing quinine, such as tonic water or lemon bitter, from the day of inoculation with the malaria challenge agent (Day -8) until after rescue medication has completed;
- Not to consume poppy seeds in the 24 hours prior to screening, day of admission for confinement for IMP administration (Day -1) and day of first dose of rescue medication;
- Not to consume food or beverages containing alcohol 24 hours prior to each alcohol breath test and for the entire period of confinement at the clinical unit for IMP administration (Days -1 to Day 5).
    - Volunteers should not drink more than 2 standard drinks per day from 24 hours prior to inoculation with the malaria challenge agent on Day -8, and after discharge from confinement until the end of treatment with rescue medication;
- Not to consume beverages containing xanthine bases (eg, Red Bull, coffee) during the entire period of confinement at the clinical unit for IMP administration (Day -1 to Day 5).
    - Volunteers should not consume more than 400 mg caffeine per day (equivalent to more than 4 cups of coffee) from inoculation with the malaria challenge agent on Day -8 until admission for confinement at the clinical unit (Day -1), and after being released from confinement at the clinical unit on Day 5 until the end of treatment with rescue medication;
- Not to use tobacco during confinement at the clinical unit for IMP administration (Day -1 to Day 5)
    - Volunteers should not smoke more than 5 cigarettes (or equivalent) per day until Day -1 and after being discharged from confinement at the clinical unit on Day 5 until the end of treatment rescue medication.
- Abstain from strenuous exercise sessions for 4 days prior to IMP administration until Day 14 post IMP.

4.3.4 Screen Failures

Unscheduled visits may be planned to assess, confirm, and follow-up on out-of-range clinical laboratory test, vital sign, or ECG values that determine a volunteer's eligibility. A positive urine drug screen should only be re-tested if there is a strong rationale for doing so (for example, false positive). The result of the re-test must be considered for volunteer eligibility and must be available prior to inoculation with the malaria challenge agent or IMP administration. Findings made during unscheduled visits should be reported in the source document.

If a volunteer does not meet all selection criteria (is a screen failure) but at some point in the future is expected to meet the eligibility criteria, the volunteer may be rescreened on one occasion only. Volunteers who are rescreened will undergo the informed consent process, be assigned a new volunteer number, and then restart a new screening phase.

Minimum information to be collected on screen failures include demography, details of the screen failure, eligibility criteria and any SAE. Screen failure information will not be entered into the eCRF. Volunteers who fail screening due to an underlying medical condition (acute self-limiting viral infections do not apply) previously unknown to them

will be reimbursed for their time, and provided with the appropriate referrals for guidance and counselling for their condition.

4.3.5 Withdrawal of Volunteers

Volunteers are free to withdraw from participating in the trial at any time upon request and irrespective of the reason. The Principal Investigator or delegate will endeavour to obtain the reason for withdrawal and will ask the volunteer to attend an early termination visit where safety procedures planned for EOS will be conducted (including PK sample if appropriate).

The Sponsor and/or Principal Investigator or delegate may discontinue or withdraw a volunteer from the trial for the following reasons:

- Pregnancy in a female volunteer (must be withdrawn from the study)
- Significant non-compliance or major protocol deviation
- Any clinical AE, laboratory abnormality or other medical condition or situation occurs that would not be in the best interest of the volunteer to continue to participate in the study (see also Section 4.3.8 for stopping rules)
- An exclusion criterion newly develops or was not previously recognized that precludes the volunteer from continuing to participate in the study;
- Participating in any other investigational product study while enrolled in this study

The reason for volunteer discontinuation or withdrawal from the study will be recorded on the CRF and withdrawn volunteers will be requested to attend an early termination visit (safety procedures are the same as for EOS).

The Principal Investigator or delegate will continue to provide medical care for any SAEs with the volunteer's permission, until symptoms resolve and/or the volunteer's condition becomes stable.

4.3.5.1 Withdrawn/discontinued Inoculated Volunteers

Volunteers who have been inoculated with the malaria challenge agent and wish to withdraw or are withdrawn from the trial, must complete anti-malarial rescue treatment as described in Section 5.3. Parasitaemia must be confirmed negative by a minimum of two negative malaria 18S qPCR tests.

The Principal Investigator or delegate will demonstrate due diligence in reminding the volunteer of the importance of completing anti-malarial treatment and in following up with the volunteer to ensure treatment was completed successfully.

After anti-malarial rescue treatment, the volunteer will be asked to attend an early termination visit voluntarily after two negative malaria 18S qPCR tests (safety procedures are as described for EOS).

4.3.5.2 Replacement of Withdrawn/discontinued Volunteers

Volunteers who have signed the informed consent form but have not received the trial intervention (inoculation with the malaria challenge agent) may be replaced.

The replacement of volunteers who have received any trial intervention (inoculation with the malaria challenge agent only or inoculation and IMP) and withdraw or are withdrawn or discontinued from the trial, must be discussed between the Principal Investigator and the Sponsor.

In each cohort, if more than two discontinuations due to non-safety related reasons occur, additional subjects may be recruited to replace the discontinued subjects on agreement with the study Sponsor.

4.3.6 Lost to Follow-Up

The Principal Investigator or delegate will make every effort to contact volunteers who fail to return to the clinical site for visits scheduled in the protocol. All efforts must be recorded in source document.

4.3.6.1 Inoculated Volunteers Lost to Follow-up

For volunteers who have been inoculated with the malaria challenge agent and have not received any anti-malarial rescue treatment, follow-up is critical as they will have blood-stage malarial parasites. A volunteer will be deemed 'missing' if they do not reply to communication to their personal mobile phone and the nominated contact's phone number for more than 24 hours. The Principal Investigator or delegate will organise a visit to the volunteer's home if they are unable to be contacted for more than 36 hours, and if the volunteer is still absent the Principal Investigator or delegate will notify the local police for assistance in locating the missing volunteer.

Once the volunteer has been found and still has parasitaemia, then the volunteer will receive rescue treatment as described in Section 5.3 at the Principal Investigator's or delegate's discretion and in agreement with the Medical Monitor. An ET visit should be scheduled after two negative malaria 18S qPCR results as per Section 4.3.5.1.

4.3.7 Study Discontinuation

The Sponsor, Principal Investigator, approving HREC, and regulatory authorities independently reserve the right to discontinue the trial at any time for safety or other reasons. Where practical, this will be done in consultation with the Sponsor and all parties notified in writing where applicable. The Sponsor and Principal Investigator will ensure that volunteers' interest and safety are protected, and the Principal Investigator must review all volunteers and complete all records as required.

4.3.8 Trial Intervention/Treatment Discontinuation – Stopping Rules

The Sponsor, Principal Investigator, approving HREC, and regulatory authorities independently reserve the right to discontinue the trial at any time for safety or other reasons. Where practical, this will be done in consultation with the Sponsor and all parties notified in writing where applicable. The Sponsor and Principal Investigator will ensure that volunteers' interest and safety are protected, and the Principal Investigator must review all volunteers and complete all records as required.

In the event of premature trial termination or suspension, the above-mentioned parties will be notified in writing by the terminator/suspender stating the reasons for early termination or suspension (with the exception of the Sponsor's responsibility for notifying the Regulatory Authorities). After such a decision, the Sponsor and the Principal Investigator will ensure that adequate consideration is given to the protection of the volunteers' interests and safety. The Investigator must review all volunteers as soon as practical and complete all required records.

At study entry and prior to inoculation, a full eligibility check will be performed. Volunteers who do not meet the study protocol inclusion/exclusion criteria will not be inoculated.

4.3.8.1 Guidance for Rescue Medication instead of IMP on Day 1

Once inoculated, it is expected that some study participants will experience malaria symptoms and biological changes (i.e. mild ALT/AST elevation, mild decrease in neutrophil count). Therefore, the following criteria should be considered as guidance for the decision not to proceed with IMP dosing on Day 1 and preference for rescue medication instead:

1. A volunteer experiences an SAE or ≥Grade 3 event (including inoculum-related events such as neutropenia or ALT/AST elevations).
2. A volunteer with positive alcohol or urine drug screen.
3. A volunteer with one of the following ECG abnormalities:
   - QTcB or QTcF at any time >480 msec,
   - Bundle branch block (except right bundle branch block that was present prior to IMP administration),
   - Any arrhythmia, except:
     a. *Sinus bradycardia that is clinically asymptomatic, and not associated with any other relevant ECG abnormalities,*
     b. *sinus tachycardia that is clinically asymptomatic, and not associated with any other relevant ECG abnormalities,*
     c. *Respiratory sinus arrhythmia,*
     d. *Wandering atrial pacemaker,*
     e. *Isolated, single premature atrial/ventricular complex (i.e., no bigeminy, trigeminy, couplets, triplets or salvos) that does not occur more than once in a particular ECG tracing.*
4. The Investigator and delegates may also decide not to proceed with IMP dosing based on other safety signals (clinical, ECG, vitals and laboratory tests) not included in the above criteria if there is a risk perceived for the study volunteers of possibly receiving a subtherapeutic dose of MMV533.

In each cohort, if more than two discontinuations due to non-safety related reasons occur, additional subjects may be recruited to replace the discontinued subjects on agreement with the study Sponsor.

4.3.8.2 Toxicity Criteria and Progression From Cohort 1 to Cohort 2

For progression from Cohort 1 to Cohort 2, the SRC members will include at least the Medical/Project Monitor, the Medical Director, the PI and an infectious diseases physician with expertise in malaria (or their delegates). The MMV Project Director or further internal or external experts such as a clinical lead and/or a statistician may be consulted by the SRT as necessary.

An SRC meeting is scheduled to occur after completion of Cohort 1 prior to commencing Cohort 2. The SRC will review available Cohort 1 safety/tolerability and parasitaemia data up to study Day 22 for all inoculated participants in Cohort 1 before enrolment of Cohort 2 and determine whether progression to Cohort 2 is indicated. If no safety concerns are identified during SRT review, progression to Cohort 2 will take place.

Initiation of Cohort 2 will be put on hold and further review will be conducted by the SRT, if any of the following toxicity criteria are observed in Cohort 1:

- Inoculum-related or IMP-related SAE; or

- any other critical Inoculum-related finding that may place participants at risk within the same cohort or in the next cohort; or
- two or more IMP-related severe (grade 3 or higher), same organ class AEs.

If after data review the SRC confirms that any of the above has been met, including a relationship with the Inoculum and/or IMP, progression to Cohort 2 might not be conducted.

4.4 RISK/BENEFIT ASSESSMENT OF STUDY TREATMENTS AND INTERVENTIONS OTHER THAN IMP

4.4.1 Malaria Challenge Agents and the IBSM

Potential risks to volunteers from inoculation with the malaria challenge agent have been identified through review of the literature and previous clinical studies conducted to date using the IBSM model with *Plasmodium falciparum* isolates. Further information can be found in the Investigator's Brochure for the 3D7 malaria challenge agent.

Briefly, the *Plasmodium falciparum* 3D7 inoculum has been used to challenge 401 healthy volunteers in 32 IBSM Phase 1 clinical trials, 27 of which were successfully undertaken at the clinical site (Q-Pharm). Risks include development of mild/moderate symptoms associated with malaria infection following the challenge, adverse effects of the IMP and adverse effects of the approved malaria rescue medications artemether/lumefantrine (Riamet® 20 mg/120 mg), primaquine (Primacin™; if required), Malarone® (if required) and artesunate (if required). Risk management of other blood borne infections, transfusion reactions, liver function abnormalities and cardiac AEs are outlined below.

4.4.1.1 Risk management of blood borne infections

The malaria challenge agent used to inoculate the volunteers contains a very small amount of donor blood, however the risk of the volunteer being infected by a possible blood borne virus is extremely low due to:

- The blood donors were screened and tested negative for active blood borne infections,
- White blood cells were removed from the donor blood by the Australian Red Cross Blood Service, lowering the risk of infections transmitted by infusion,
- The volume of blood used in the IBSM model to transmit malaria to the volunteers is many thousands of times smaller than the volume of blood used in a blood transfusion.

As part of the safety monitoring for this study, all volunteers will provide a serum safety sample before being inoculated with the malaria challenge agent and at the end of study visit (EOS). To date, no blood borne infections have been reported in any of the 401 volunteers who have been inoculated with the *P. falciparum* 3D7 malaria challenge agent in IBSM studies.

4.4.1.2 Risk management of reaction to the blood sample

It is possible that volunteers could suffer a transfusion reaction after being inoculated with the malaria challenge agent, or develop alloantibodies to the donor red blood cells (RBCs) that may make blood transfusion more difficult for that volunteer in the future. The risk of developing such alloantibodies is considered extremely low as the donor blood used to produce the 3D7 malaria challenge agent was blood group O Rh(D) negative which is generally considered to be a 'universal donor'. Recipients transfused with such blood are considered to have minimal risk of developing RBC alloantibodies, and the volume of

blood used in a typical blood transfusion is much larger than that present in the malaria challenge agent used in the IBSM model. To date, one volunteer developed an antibody response to a minor Rh antigen (anti-E antibody) after being inoculated with 3D7 malaria challenge agent, however there was no laboratory evidence that the donor RBCs in the malaria challenge agent provoked production of the alloantibody.

As part of the safety monitoring for this study, volunteers will be tested for RBC alloantibodies at screening and at the end of the study, and will be monitored for signs and symptoms of transfusion reactions immediately after being inoculated with the malaria challenge agent.

4.4.1.3 Risk management of malaria infection

The number of viable blood stage parasites in the malaria challenge agent used to infect the volunteers in this study is approximately 2,800. This is substantially lower than the approximately 30,000 parasites released into the blood after breaking out of a single liver cell after the bite of a single malaria-infected mosquito. In this study, parasite growth and malaria symptoms will be closely monitored after volunteers are inoculated with the malaria challenge agent. Parasite growth will be quantitatively measured using quantitative polymerase chain reaction (qPCR) to assay for malaria genome copies.

The IMP will be administered 8 days after inoculation with the malaria challenge agent, as this is prior to the time at which advanced clinical symptoms of malaria are likely to occur based on the previous clinical trials performed with the 3D7 malaria challenge agent. All volunteers will receive compulsory standard anti-malarial treatment by Day 21 ±3, or earlier if required. Refer to the 3D7 Investigator's Brochure for further information.

4.4.1.4 Risk management of liver function abnormalities

Some liver function test (LFT) abnormalities have been reported in volunteers participating in IBSM studies:[4]

- Transient asymptomatic LFTs including rare cases of alanine aminotransferase (ALT)/aspartate aminotransferase (AST) elevations >10 fold the upper limit of normal (ULN)
    - No changes in bilirubin reported except in one volunteer with unappreciated pre-existing liver disease
    - Did not require treatment and resolved by the end of the studies.
- LFT elevations were considered as serious adverse events (SAEs) by two Pharma sponsors, due to their internal processes for SAE notifications.
    - Following independent review involving drug-induced liver injury experts, it was concluded that these LFT elevations in inoculated non-immune healthy volunteers were most likely due to malaria infection rather than caused by an IMP.

Safety monitoring for this study will include regular safety monitoring to assess for asymptomatic LFT abnormalities. Volunteers are required to reduce their consumption of possibly hepatotoxic substances (including alcohol and paracetamol) during the study, and drugs of abuse are not permitted.

4.4.1.5 Risk management of cardiac adverse events (AEs)

To our knowledge, four cardiac SAEs have been reported in healthy volunteers in the Netherlands participating in malaria challenge studies using sporozoites (i.e., direct feeds by infected mosquitoes rather than IBSM infection). No cardiac SAEs caused by the challenge agents have been reported in IBSM studies. However, in a recent study two

volunteers (one infected with the *P. falciparum* 3D7 challenge agent and one infected with another malaria challenge agent strain *P. falciparum* K13) developed ventricular extra systoles that were classified as moderate AEs possibly related to malaria. As a precaution, people at significant risk of cardiovascular disease will be excluded from participating in IBSM studies, and regular safety monitoring including physical examination and ECG recordings will take place for all volunteers. Follow-up with a cardiologist is also available if any cardiac AEs are seen during the IBSM studies.

4.4.1.6 Risk/benefit of inoculation with malaria challenge agent

The clinical study has been designed such that the risk to volunteers in this study will be minimized by adequate selection of eligibility criteria (including red blood cells alloantibodies tested at screening) and schedule of clinical monitoring, in-house observation, and administration and treatment duration.

There are no direct benefits to the volunteer, however there may be a benefit to society by helping to develop future antimalarial therapies.

4.4.2 Malarial Rescue Medication/s

Information on the potential risks and adverse effects of rescue medications Riamet®, Primacin™, Malarone® and Artesunate can be found in the respective product/consumer information documents. To mitigate the potential risks, the wellbeing of volunteers during and after administration of rescue treatment will be appropriately monitored. Volunteers who have any known contra-indication to any of the rescue medications according to the applicable labelling at screening will be excluded from participating in the study.

5 TRIAL INTERVENTIONS/TREATMENTS

5.1 INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

MMV533 is defined as the IMP for this study. Refer to the Investigator's Brochure for further information. Refer to the Pharmacy Manual for further information on the required handling of the IMP.

Volunteers will be admitted to the clinical unit for oral dosing of the IMP.

5.1.1 MMV533

- Volunteers will fast overnight for at least 10 hours.
- Volunteers will take a single oral dose of IMP with 240 mL of water. The dose of IMP will depend on their cohort.
- Volunteers will then:
    - Be requested to not drink liquids for 1 hour;
    - Remain seated for 10 minutes, then stay in a semi-recumbent position for the next 2 hours (except during assessments that require lying positions);
    - Not be permitted food for at least 4 hours.
- Lunch and dinner will be provided approximately at 5 hours and 12 hours post IMP administration, with snacks available between meals. Daily water supply should not exceed 3L.

5.2 IBSM MODEL MALARIA CHALLENGE AGENT

The malaria challenge agent is composed of approximately 2800 viable human erythrocytes infected with the *Plasmodium falciparum* 3D7 parasite, and is administered intravenously to the volunteer as part of the IBSM model of controlled infection. The

infected erythrocytes are from a master cell bank (MCB) that has been well-characterised and used in a significant number of IBSM studies. The 3D7 parasite from this MCB is sensitive to artemisinin treatment. Refer to the Investigator's Brochure for the 3D7 malaria challenge agent for further information.

Volunteers will attend the clinical unit on the morning of Day -8 to be inoculated with the malaria challenge agent as follows:

- Volunteers may have food until at least 30 minutes prior to inoculation with the malaria challenge agent.
- Volunteers will be cannulated with an appropriate gauge cannula, the placement and patency of which will be checked by flushing the vein with clinical grade saline.
- A single syringe of the prepared malaria challenge agent containing approximately 2800 viable human erythrocytes infected with *P. falciparum* 3D7 parasite in 2 mL of saline will be injected and the cannula flushed with 5-10 mL of clinical grade saline.
- The actual number of parasites will take into account the loss of viability resulting from cryopreservation, storage and thawing.
- All volunteers attending on the same day will be inoculated within a 60 minute period and within 4 hours of preparation of the malaria challenge agent. The expiry time will be listed on the inoculum label.
- After removal of the cannula, haemostasis will be ensured by use of appropriate dressing.
- Volunteers will remain at the clinical site for at least 60 minutes after inoculation with the malaria challenge agent for safety monitoring.

5.3 MALARIA RESCUE MEDICATIONS

All volunteers participating in the study that have been inoculated with the malaria challenge agent (including inoculated volunteers who withdraw or are withdrawn from the study – see Section 4.3.5.1) will receive mandatory malarial rescue treatment.

5.3.1 Riamet® (Artemether-lumefantrine)

Riamet® is a standard treatment for uncomplicated malaria due to *P. falciparum* in adults. A course of Riamet® will be administered to all volunteers who have been inoculated with the malaria challenge agent including participants dosed with IMP. The standard adult dosing regimen according to the CMI will be used: 4 tablets administered orally twice daily over 3 consecutive days (6 doses at 0, 8, 24, 36, 48 and 60 hours, total course of 24 tablets). Each dose should be taken with 240 mL full-fat milk. The first dose of Riamet® will be administered to the volunteer at the clinical unit. The remainder of the doses will be taken by the volunteer at home and recorded in the diary. Volunteers will be given the CMI for Riamet® and reminded of the potential side effects. Clinical staff will contact the volunteer on a daily basis during the dosing period to confirm compliance.

5.3.2 If Gametocytaemia is suspected/confirmed

The presence of gametocytes (gametocytaemia) will be detected by parasite lifecycle stage qRT-PCR performed at the discretion of the Principal Investigator or delegate. If present, Primacin™ may be administered at the same time as the first dose of Riamet® to ensure complete clearance of gametocytes. Primacin™ is an antimalarial agent containing primaquine phosphate and is effective against the sexual forms (gametocytes) of *P. falciparum*, disrupting transmission of the disease by eliminating the reservoir from which the mosquito carrier is infected.

Dosing regimen based on G6PD status performed at study entry:

- If not G6PD deficient - volunteers will take six Primacin® tablets (the total dose of 45 mg primaquine) as a single dose with food.
- If mildly G6PD deficient - volunteers will take two Primacin® tablets (the total dose of 15 mg primaquine) as a single dose with food.

If required, volunteers will be administered Primacin™ under supervision at the clinical unit. The volunteer will be reminded of the potential side effects of Primacin™ and given the CMI.

5.3.3 If an Allergy or Contraindication to Riamet® Develops

Malarone® may be administered at the Principal Investigator's discretion if an allergy or contraindication to Riamet® develops or is suspected. Malarone® is a fixed combination product containing atovaquone and proguanil hydrochloride. Atovaquone interferes with the biosynthesis of pyrimidines by blocking the parasite mitochondrial electron transport chain, and proguanil interferes with the biosynthesis of pyrimidines by inhibiting parasite dihydrofolate reductase.

The treatment course if required will be as recommended by manufacturer for treatment of malaria:

- four tablets of Malarone® (atovaquone 250 mg, proguanil hydrochloride 100 mg) once daily orally with food for three consecutive days.

The first dose of Malarone® will be administered to the volunteer at the clinical unit. Volunteers will be given the CMI for Malarone® and reminded of the potential side effects. The remainder of the doses will be taken by the volunteer at home and recorded in the diary. Clinical staff will contact the volunteer on a daily basis during the dosing period to confirm compliance.

5.3.4 If Volunteer Vomits or Cannot Tolerate Oral Malarial Treatment

Intravenous artesunate may be used as a rescue medication if the volunteer cannot tolerate oral antimalarial rescue treatment (e.g. vomiting). Participants will not be administered IV artesunate if vomiting occurs on the second or third day of oral rescue medication.

Artesunate is an artemisinin derivative and is the recommended parenteral treatment for severe malaria in Australia. Artesunate does not have marketing approval in Australia but importation is allowed for severe malaria under the Special Access Scheme (SAS).[11] The Sponsor has sourced artesunate from Guilin Pharmaceutical (Shanghai) Co., Ltd and facilitated its importation. The manufacture of the artesunate is undertaken in a WHO Pre-Qualified GMP facility. Artesunate for IV administration is provided as a powder for reconstitution (60 mg artesunic acid) in a vial.

If required, artesunate will be administered intravenously under medical supervision at a dose of 2.4 mg/kg at approximately 0, 12, 24 hours, and then daily for up to seven days or until the volunteer is able to take oral drugs.

5.4 SELECTION OF DOSE IN THE STUDY

5.4.1 MMV533

Up to three doses for each cohort will be selected for a single oral dose of the IMP per volunteer to be taken as per Section 5.1.1.

Choice of the dose levels will be made cohort by cohort based upon a review of the safety, tolerability, and PK data of single dose level cohorts from cohort 1 and from PK and PD data from previous cohorts. The SRC will review safety and tolerability data up to Day 22, and PK and PD analysis outcomes (based on PD and PK data up to Day 15) from the previous cohort, prior to inoculation of the next cohort with the malaria challenge agent.

- Doses tested in Cohort 1:
    - The two doses tested in Cohort 1 will be predicted to cover a sub-curative effect (ie, expected to be associated with recrudescence) to allow PK and PD parameters to be calculated and the efficacious dose in patients to be estimated. This cohort will be split into 2 sequential sub-cohorts of 4 participants each:
        - Cohort 1A: the first four participants will receive a dose of MMV533 of 20 mg.
        - Cohort 1B: the highest dose of MMV533 to be tested in the 4 remaining participants enrolled in Cohort 1 will not exceed a dose tested and deemed to be safe in the FIH study and will not exceed 50 mg (i.e. Cohort 4 of the FIH study).

    There will be no safety review in-between cohorts 1A and 1B.

- Doses (up to 3) tested in Cohort 2 will depend on the results from Cohort 1:
    - The doses will be chosen so as to refine the estimation of the parameter(s) with the greatest uncertainty; that is, high doses will be favoured to increase confidence in Emax, and low doses to increase confidence in EC50. A range of doses will be considered if both Emax and EC50 have similarly large uncertainties. The highest dose of MMV533 to be tested in Cohort 2 will not exceed the highest tolerated dose documented in the FIH study or 200 mg, whichever is lower.
    - Cohort 3 will proceed if the uncertainty of one or more of the PK and/or PD parameters is still large after Cohort 2. The highest dose of MMV533 to be tested in Cohort 2 will not exceed the highest tolerated dose documented in the FIH study or 200 mg, whichever is lower.

5.4.2 Other Study Treatments/Interventions

The inoculum dose of malaria challenge agent used is as described in the *P. falciparum* 3D7 Investigator's Brochure and is based upon use in the IBSM model in previous clinical trials.

Dose regimens for malarial rescue medications are the standard dosing regimen for adults as recommended in their respective product information/CMI.

5.5 SELECTION AND TIMING OF DOSE FOR EACH VOLUNTEER

5.5.1 MMV533

The SRC will decide the doses of IMP to be used cohort by cohort, except Cohort 1A will be administered 20 mg MMV533 as per Section 5.4.1.

The study is open label. All volunteers will be administered IMP on Day 1 as described above in Section 5.1.1, eight days after inoculation with the malaria challenge agent when

parasitaemia of all volunteers is expected to be between approximately 5,000 - 25,000 parasites/mL (unless rescue medication is administered as per Section 4.3.8.1).

See Section 6.1.5 for Note on appropriate consideration of COVID-19 symptoms. IMP should not be administered if COVID-19 is suspected (including waiting for test results) or confirmed.

5.5.2 Other Study Treatments/Interventions

All eligible volunteers will be inoculated with the malaria challenge agent on Day -8 as described above in Section 5.2.

All volunteers inoculated with the malaria challenge agent will receive compulsory malarial rescue treatment as described in Section 5.3 on Day 21±3, or earlier under the following circumstances:

- Contraindication to IMP dosing (see Section 4.3.8.1)
- early withdrawal of inoculated volunteer (see Sections 4.3.5.1 and 5.3)
- If they experience an inoculum-related serious adverse event (SAE), or
- If they have a Common Terminology Criteria for Adverse Events (CTCAE) grade ≥3 AE deemed possibly related to malaria and not self-resolved or relieved with concomitant medications, or
- If after IMP dosing there is failure to clear parasites or recrudescence (see Section 6.3.2 for definition), or
- If the Principal Investigator or delegate considers it necessary for volunteer safety. In this situation, the Principal Investigator or delegate will discuss with the Malaria Expert and/or the Medical Monitor. However, antimalarial medication may be administered prior to consultation if immediate treatment is deemed necessary for volunteer safety.

In these cases, associated safety assessments must continue as per the Detailed Schedule of Activities for rescue medication in Table 3 and malaria clinical score and malaria 18S qPCR must be continued for up to a minimum of 72 hours after the first dose of malaria rescue medication.

The Principal Investigator or delegate must record the reasons for any administration of rescue medication prior to Day 21±3 in source document.

5.6 DOSE INTERRUPTIONS AND REDUCTIONS

Dose interruptions per volunteer are not applicable to the IMP as it will be administered in a single dose. Dose adjustments will be made as outlined above in Section 5.4 and as recommended on an ongoing basis cohort by cohort by the SRC.

If required, Primacin® dose may be reduced as described above in Section 5.3.2.

5.7 SUPPLY, PACKAGING AND LABELLING OF STUDY TREATMENTS

The IMP and other study medications/interventions (non-IMP) will be manufactured and packaged according to Good Manufacturing Practice (GMP), all local regulations and labelled for clinical trial use in accordance with Australian requirements including Annex 13 of GMP. The IMP will be supplied to the clinical unit with an acknowledgement of receipt form.

5.7.1 MMV533

MMV533 is supplied by Piramal (Pharmaceutical Development Services, Piramal Enterprises Ltd. Ahmedabad) and is stored at PCI Pharma Services, Port Melbourne prior to delivery to site.

MMV533 will be provided to site as film-coated tablets in two strengths: 5 mg and 50 mg (identical in shape and size). The tablets are white to off-white, round, biconvex, film-coated tablets.

5.7.2 Malaria Challenge Agent

The *P. falciparum* 3D7 MCB was produced from an individual with blood group ORh(D) negative who was infected with the parasite by mosquito bite. The 3D7 MCB was cryopreserved, aliquoted into cryovials, and is stored in liquid nitrogen under controlled conditions at QIMR Berghofer.

On the day of inoculation, the malaria challenge agent will be prepared aseptically from a frozen cryovial of the *P. falciparum* 3D7 MCB at QIMR. Preparation includes thawing, washing and resuspending the infected red blood cells in saline to a final volume of 2 mL, and the malaria challenge agent will then be supplied without delay to the clinical unit as single doses in syringes. The syringes will be labelled in accordance with GCP guidelines and local regulatory requirements, including the expiry time for use. All volunteers should be inoculated prior to the expiry of the malaria challenge agent (within 4 hours of preparation; expiry time will be listed on inoculum label).

5.7.3 Malarial rescue medications

Riamet®, Malarone®, and Primacin™ will be sourced and provided by the clinical unit:

- Riamet® will be supplied as tablets containing 20 mg arthemether and 120 mg lumefantrine.
- Malarone® will be supplied as tablets containing 250 mg atovaquone and 100 mg proguanil hydrochloride.
- Primacin™ will be supplied as tablets containing 13.2 mg primaquine phosphate equivalent to 7.5 mg primaquine.
- Artesunate for intravenous administration is provided as a powder (60 mg artesunic acid) for reconstitution in a vial.

5.8 STORAGE OF STUDY TREATMENTS

Prior to dispensing, the IMP and malarial rescue treatments must be stored in a secure and locked storage area with limited access, and under monitored, temperature controlled conditions as appropriate. All storage requirements are detailed in the respective Investigator Brochures or product information/CMI.

The Principal Investigator or delegate (e.g. clinical unit Pharmacist) will be responsible for the correct storage and handling of IMP and rescue medications, and for the malaria challenge agent when received on the day of use. Deviations from the storage requirements, including corrective action, must be documented. Refer to the Pharmacy Manual for further information.

5.9 ACCOUNTABILITY, RECONCILIATION AND RETURN OF THE STUDY TREATMENTS

The Principal Investigator will ensure that the IMP, malaria challenge agents and malarial rescue medication/s are dispensed only to eligible volunteers enrolled in this study and

will ensure that complete and current dispensing and inventory records are maintained. The site's dispensing logs must record every episode of dispensing of the IMP, malaria challenge agent and malarial rescue medication/s.

The logs must contain the following information:

- Date of receipt.
- Number of tablets / syringes / vials received.
- Batch number(s).
- The identification of the volunteer to whom the tablets / syringes / vials was dispensed.
- The date(s), time and quantity dispensed to the volunteer.
- The cumulative total of malaria challenge agents, IMP and rescue medication at site.
- Tablets / syringes / vials damaged, destroyed, or returned.

Supplies of the IMP will be shipped to the clinical unit prior to study start. The study monitor will perform drug accountability during routine monitoring visits. The malaria challenge agent will be provided to the clinical unit on the day of inoculation as required.

Once the study has completed or has been discontinued, final accountability and reconciliation will be performed. Any discrepancies will be investigated and the resolution documented. All full, partially full, and empty containers of IMP and other medication must be returned to the Pharmacy, Manufacturer or Sponsor for destruction, and the appropriate form sent to the Sponsor. Any remaining unused malaria challenge agent will be discarded as per approved standard operating procedures.

Please refer to the Pharmacy Manual for further details on the storage, handling, and dispensing of IMPs, malaria challenge agents and rescue medications.

5.10 PROHIBITED CONCOMITANT THERAPY

Any medication that is exclusionary for eligibility at Screening remains prohibited during the study. Volunteers will be requested to refrain from taking non-approved concomitant medications during their participation in the study.

Ibuprofen may be permitted for use at doses up to 1.2 g/day or paracetamol at doses up to 2 g/day only after the volunteers discusses with the Principal Investigator or delegate. Ibuprofen is the preferred treatment, however paracetamol may be used if ibuprofen does not relieve the volunteer's symptoms. Acetyl salicylic acid and/or diclofenac may also be permitted after discussion with the Principal Investigator or delegate. Contraceptives are permitted.

The use of any concomitant medication other than ibuprofen, paracetamol, acetyl salicylic acid or diclofenac as described above will only be permitted if specified in the inclusion criteria, study procedures (such as malaria rescue treatment) or otherwise required medically. Any concomitant use of medications other than study-specific treatments should be recorded by the volunteer in the diary card.

Any use of concomitant medication including name of medication, daily dosage and duration of use must be recorded by study staff in source documents and in the eCRF.

5.11 TREATMENT COMPLIANCE

Volunteers will be administered a single oral dose of IMP under medical supervision while confined at the clinical unit. Volunteers will be inoculated with the malaria

challenge agent by clinical unit study staff while attending the clinical unit. Treatment compliance for volunteers administered malarial rescue medication is described in Section 5.3 above.

5.12 MEASURES TO MINIMIZE BIAS

5.12.1 Randomisation Procedures

The study is open label and randomised. Each cohort will be composed of up to 3 dose groups with a minimum of 2 subjects per dose group. If more than one dose level are tested in a given cohort, subjects will be randomised after inoculation day but prior to Day 8 and administered a single oral dose of MMV533. The randomisation schedule will be generated by a statistician using a validated system as described in a Randomisation Plan and/or Randomisation Procedure.

6 STUDY ASSESSMENTS AND PROCEDURES

6.1 STUDY CONDUCT SCHEDULE

The schedules for all assessments and study activities are summarised in the Schedule of Activities and shown in more detail in the Detailed Schedule of Activities in Section 1.3. All study assessments and procedures are as described in Section 6.2, 6.3 and Section 7.

Protocol waivers or exemptions are not permitted.

6.1.1 Screening

The screening period for the study will be 25 days from Day -36 to Day -12. During this time, the volunteer will sign the patient information consent form (PICF) prior to any study-related procedures or assessments and eligibility will be assessed (see Schedule of Activities Table 1).

Demographics and comprehensive medical history will be documented at screening.

6.1.2 Inclusion/Eligibility Visit

Eligibility must be confirmed prior to inoculation with the malaria challenge agent on Day -8.

The volunteer will attend the clinical unit at least once between Days -11 to -9 in order to assess eligibility criteria that require substantial time for results prior to inoculation with the malaria challenge agent.

Samples for clinical laboratory testing will be obtained at this visit to ensure results are available prior to inoculation on Day -8. A second visit is permitted if reassessment is required to confirm eligibility prior to inoculation.

Only one re-test of any baseline parameter is permitted, and the result must be available for review prior inoculation with the malaria challenge agent.

In addition, the following will be performed:

- Any new medical conditions, illnesses and concomitant medications since screening should be checked for and recorded.
- Symptom-directed physical examination will be performed if required.
- Body weight will be re-checked.
- Only if participant has consented to optional QIMR study as per Section 7.6: collect blood sample for QIMR research serum samples

For each cohort, eligible additional volunteers may be available as back-up, in the event planned cohort volunteers withdraw or are withdrawn prior to inoculation with the malaria challenge agent.

Volunteers who meet all of the inclusion criteria and none of the exclusion criteria will be eligible for inclusion into the study and to be inoculated with the malaria challenge agent on Day -8. Volunteers will be reminded of the lifestyle considerations for the duration of the study (see Section 4.3.3).

6.1.3 Day -8 Inoculation with Malaria Challenge Agent

Volunteers will attend the clinical unit on the morning of Day -8 to be inoculated with the malaria challenge agent. The Principal Investigator or delegate will review all eligibility information for each volunteer prior to inoculation with the malaria challenge agent, including review of clinical laboratory safety tests performed at the inclusion/eligibility visit (see Section 6.1.2) and assessments conducted pre-inoculation on Day -8.

For each cohort, eligible additional volunteers may be available as back-up, in the event planned cohort volunteers withdraw or are withdrawn prior to inoculation with the malaria challenge agent.

The Principal Investigator or delegate will emphasise to the volunteer the importance of returning to the clinical unit for IMP administration and subsequent mandatory malaria rescue treatment after inoculation with the malaria challenge agent. The volunteer will be asked to confirm that they will not be living alone from Day -8 until EOS and to re-confirm the contact details of housemates recorded at the screening visit.

The following will be performed prior to inoculation with the malaria challenge agent:

- Check for and record any new medical conditions, illnesses and concomitant medications since screening and eligibility visit,
- Alcohol breath test and urine drug screen,
- Symptom-directed physical examination if required,
- Vital signs and body temperature,
- Triplicate 12-lead ECG,
- Cannulate volunteer with indwelling intravenous cannula into peripheral vein of volunteer's forearm to obtain blood samples and administer the malaria challenge agent (record which arm used),
- Calculate malaria clinical score,
- Collect blood samples for malaria 18S qPCR, inoculum-related safety serum sample and IMP safety serum sample.
- Confirm eligibility.

The volunteer will be inoculated with the malaria challenge agent as described in Section 5.2.

The following will be performed after the volunteer is inoculated with the malaria challenge agent:

- Monitor the volunteer for at least 60 minutes after inoculation to check for immediate adverse reactions, and record any AEs and/or concomitant medications,
- Educate volunteers on the signs and symptoms of malaria (see Appendix 2: Malaria Clinical Score);

- Inform volunteers that they should immediately contact the Principal Investigator or delegate if they experience any unexpected symptom, effect or event and that they can contact the clinical unit by telephone 24 hours a day, and:
- Provide volunteer with a volunteer card listing the study number and appropriate clinical unit telephone contact details. The volunteer must also provide telephone contact details so that they can be contacted in an emergency.
- Emphasise to volunteers the importance of returning to the clinical unit on the nominated day (Day -1) or as advised by the clinical unit staff, for IMP administration as antimalarial treatment,
- Issue diary card and thermometer to volunteers to collect information during outpatient periods including oral temperature (if required), symptoms of malaria, and details of daily physical activity and alcohol consumption. Volunteers will bring their diary cards to each visit to the clinical trial unit for review. Site staff will enter the diary information into the eCRF.
- Measure vital signs approximately 60 minutes after inoculation with the malaria challenge agent and prior to leaving the clinical unit.
- Calculate malaria clinical score prior to leaving the clinical unit.

6.1.4 Days -7 to -5 Malaria monitoring by phone

Volunteers are expected to be asymptomatic from Days -8 to -5. Clinical unit staff will ring or text message the volunteer daily to monitor well-being and solicit AE information. Details of this contact will be recorded in the source document.

Volunteers will attend the clinical unit if any AEs are reported during this period involving assessments of vital signs, body temperature, 12-Lead ECG, symptom directed physical examination, malaria clinical score and possible blood sampling for 18S qPCR.

After inoculation with the malaria challenge agent, participants may develop symptoms of malaria (e.g. fever) that are also symptoms of COVID-19. The risk of COVID-19 is currently low in Queensland. However, the Investigator will consider the possibility of COVID-19 when unexpected symptoms (e.g. sore throat, anosmia, cough/respiratory symptoms) are reported along with fever. The Investigator will review all symptoms, their timing with respect to date of inoculation, 18S qPCR results and other available information to decide if symptoms can be confidently attributed to malaria/inoculation. If the symptoms are considered consistent with malaria/inoculation, and at the discretion of the Investigator, the site standard procedures for participants with COVID-19 symptoms will not be required. This decision will be documented in source notes by the Investigator. However, if considered appropriate or necessary by the Investigator, the participant may be isolated at the clinical unit for COVID-19 testing and quarantine as per site procedure, and to ensure safety monitoring for malaria is performed as per protocol.

6.1.5 Day -4 until Day -1 Outpatient Visits

NOTE for consideration of COVID-19 symptoms: After inoculation with the malaria challenge agent, participants may develop symptoms of malaria (e.g. fever) that are also symptoms of COVID-19. The risk of COVID-19 is currently low in Queensland. However, the Investigator will consider the possibility of COVID-19 when unexpected symptoms (e.g. sore throat, anosmia, cough/respiratory symptoms) are reported along with fever. The Investigator will review all symptoms, their timing with respect to date of inoculation, 18S qPCR results and other available information to decide if symptoms can be confidently attributed to malaria/inoculation. If the symptoms are considered consistent with malaria/inoculation, and at the discretion of the Investigator, the site

standard procedures for participants with COVID-19 symptoms will not be required. This decision will be documented in source notes by the Investigator. However, if considered appropriate or necessary by the Investigator, the participant may be isolated at the clinical unit for COVID-19 testing and quarantine as per site procedure, and to ensure safety monitoring for malaria is performed as per protocol.

Volunteers will attend the clinical unit for outpatient monitoring visits once (in the morning) on Day -4 and twice daily on Days -2 and -3 (separated by 12±2 hours on each day). The following procedures will be performed:

- Symptom-directed physical examination when signs or symptoms malaria are identified and it is clinically indicated (once daily only),
- Elicit information on AEs and concomitant medications and review diary card,
- Vital signs and body temperature,
- Standard 12-lead ECG on Day -1 morning only,
- Collect blood samples for LFTs only on Day -3,
- Calculate malaria clinical score,
- Collect blood sample for malaria 18S qPCR.

Prior to IMP dosing, the Investigator and/or delegates should ensure that no contraindication to administration of IMP are present as described in Section 4.3.8 (in which case, rescue medication should be administered instead of IMP).

6.1.6 Day -1 to Day 5 Confinement for IMP administration

Volunteers will be admitted to the clinical unit in the morning of Day -1 for confinement and observation for at least 108 hours after IMP administration. Parasitaemia is expected to be approximately between 5,000 and 25,000 parasites/mL.

See Section 6.1.5 for Note on appropriate consideration of COVID-19 symptoms. IMP should not be administered if COVID-19 is suspected (including waiting for test results) or confirmed.

Admission procedures on Day -1:

- Symptom-directed physical examination
- Alcohol breath test and urine drug screen
- Review diary temperature card and elicit information on AEs and concomitant medications
- Vital signs and body temperature
- Cannulate volunteers with indwelling intravenous cannula into peripheral vein of the volunteer's forearm to obtain blood samples (the cannula will be locked with a mandrel between blood samples).
- Collect blood samples for haematology and biochemistry on Day -1 morning. NOTE: the results of this safety testing must be available for review prior to IMP administration on Day 1,
- Collect urine sample for urinalysis Day -1 morning, NOTE: the results of the Day -1 sample must be available for review prior to IMP administration on Day 1;
- Calculate malaria clinical score
- Collect blood sample for malaria 18S qPCR AM and PM (parasite clearance baseline sample)

Day 1 Pre-dose activities:

- Vital signs supine
- 12-lead ECG
- Body temperature
- Blood samples for:
    - haematology, biochemistry
    - PK sample
    - Malaria 18S qPCR
    - Only if participant has consented to optional QIMR study as per Section 7.6: collect blood sample for QIMR research serum samples
- Urine sample for urinalysis
- Malaria clinical score

The volunteer will be administered IMP on Day 1 as described in Section 5.1.1.

After administration of IMP, the following will be conducted:

- Follow up volunteers as inpatients for at least 108 hours after IMP administration to monitor safety, tolerability and adequate clinical response of the IMP treatment,
- Symptom-directed physical examination if signs or symptoms of malaria are identified and it is clinically indicated,
- Vital signs and body temperature at timepoints defined in the Detailed Schedule of Activities in Table 2 and Table 3 and as per Section 7.2,
- Standard 12-lead ECG at timepoints defined in the Detailed Schedule of Activities in Table 2 and Table 3 and as per Section 7.3,
- At timepoints defined in the Detailed Schedule of Activities in Table 2 and Table 3, collect blood samples for
    - clinical laboratory safety tests (Section 7.5.1)
    - PK analysis (Section 6.4)
    - Malaria 18S qPCR (Section 6.2.1)
- Collect urine for urinalysis at timepoints defined in the Detailed Schedule of Activities in Table 2 and Table 3 and Section 7.5.6.
- Calculate malaria clinical score at timepoints defined in the Detailed Schedule of Activities in Table 2 and Table 3 and Section 7.4.
- Record AEs and concomitant medications.

Before discharge at least 108 hours post-dose, the following will be conducted:

- Symptom-directed physical examination if required. Before discharge, the clinical unit staff must confirm with the Principal Investigator or delegate if any volunteer requires symptom-directed physical examination.
- Vital signs and body temperature,
- Collect blood samples for
    - Malaria 18S qPCR
    - qRT-PCR pfs25 at the discretion of the Principal Investigator or delegate (Section 6.2.1
- Calculate malaria clinical score
- Record AEs and concomitant medications

Volunteers will be permitted to leave the clinical unit 108 hours after IMP administration at the discretion of the Principal Investigator or delegate if:

- they are asymptomatic,
- have normal physical examination if a symptom directed physical examination was required,
- no clinically significant laboratory abnormalities.

6.1.7 Post-confinement Outpatient Monitoring Visits

After discharge from the clinical unit, volunteers should return morning and evening (approximately 12 hours apart) until parasite counts are <~500 parasites/mL or at the Investigator's discretion. Volunteers may then have daily visits at the Investigator's discretion. When parasite counts are <~200 parasites/mL, sampling may revert to alternate day visits at the Investigator's discretion. Once the malaria 18S qPCR results are negative, or low and stable, volunteers may be reviewed 3 times per week at the Investigator's discretion until treatment with rescue medication.

The following procedures will be conducted at each outpatient visit:

- Review diary temperature card, record AEs and concomitant medications,
- Symptom-directed physical examination if signs and symptoms of malaria are identified and it is clinically indicated,
- Vital signs and body temperature,
- Collect blood samples for malaria 18S qPCR (Section 6.2.1) on a daily basis in the morning or twice daily depending on parasite count or at the discretion of the Principal Investigator or delegate,
- Collect blood sample for qRT-PCR pfs25 at the discretion of the Principal Investigator or delegate (Section 6.3.1),
- Collect blood samples for parasite drug resistance analysis at the discretion of the Principal Investigator or delegate (Section 6.3.2).
- Calculate malaria clinical score while malaria counts are positive or at the discretion of the Principal Investigator or delegate (Section 7.4).

Outpatient visits are required at the timepoints defined in the SOA Table 3 and relevant sections when required for the following:

- Standard 12-lead ECG (Section 7.3)
- Collect blood samples for
    - clinical laboratory safety tests (Section 7.5.1)
    - PK analysis (Section 6.4)
- Collect urine for urinalysis at timepoints (Section 7.5.6).
- Only if participant has consented to optional QIMR study as per Section 7.6: collect blood sample for QIMR research serum samples (see SOA Table 3)

See Section 6.1.5 for Note on appropriate consideration of COVID-19 symptoms.

6.1.8 Mandatory Malaria Rescue Medication

All volunteers will attend the clinical unit on Day 21±3 to begin mandatory malaria rescue medication as described in Section 5.3. Malaria rescue medication may be administered earlier as described in Section 5.5.2. In these cases, associated safety assessments must continue as per the Detailed Schedule of Activities for rescue medication in Table 3 and

malaria clinical score and malaria 18S qPCR must be continued for up to a minimum of 72 hours after the first dose of malaria rescue medication.

Volunteers should take at least the first dose of rescue treatment under supervision at the clinical unit, and may take the remaining doses (if applicable) at home as determined by the Principal Investigator or delegate. Clinical unit staff will contact the volunteers on a daily basis to ensure compliance with the malaria rescue medication dosing regimen and to check well-being.

The following procedures will be performed prior to the first dose of malaria rescue medication:

- Review diary card, record AEs and concomitant medications,
- Symptom-directed physical examination,
- Alcohol breath test and urine drug screen,
- Vital signs and body temperature,
- Standard 12-lead ECG,
- Collect blood samples for
    - clinical laboratory safety tests
    - PK analysis (Section 6.4)
    - Serology (Section 7.5.3)
    - Red blood cell alloantibodies
    - Malaria 18S qPCR
    - qRT-PCR pfs25 (if required)
- Calculate malaria clinical score while malaria counts are positive or at the discretion of the Principal Investigator or delegate.

See Section 6.1.5 for Note on appropriate consideration of COVID-19 symptoms.

6.1.9 Unscheduled Monitoring Outpatient Visits

Volunteers may attend unscheduled visits at the clinical unit for malaria 18S qPCR or safety monitoring if required and at the discretion of the Principal Investigator or delegate, based on parasitaemia, clinical symptoms and/or clinical laboratory safety test results. The clinical site staff will contact the volunteer by phone to arrange these visits. Unscheduled visits will be recorded in source document and in the eCRF.

6.1.10 Day 28±3 EOS

Volunteers will attend the clinical unit for the EOS only after completion of malaria rescue medication and 2 negative malaria 18S qPCR results. The following procedures will be conducted:

- Review diary card and record AEs and concomitant medications
- Full physical examination
- Vital signs and body temperature
- Standard 12-lead ECG
- Collect blood samples for
    - clinical laboratory safety tests – may include CRP at the discretion of the Principal Investigator or delegate (Section 7.5.1)
    - Serology (Section 7.5.3)
    - Red blood cell alloantibodies
    - Malaria 18S qPCR
    - PK analysis (Section 6.4)

  - qRT-PCR if required
  - Inoculum-related Safety serum sample and IMP safety serum sample
  - Only if participant has consented to optional QIMR study as per Section 7.6: collect blood sample for QIMR research serum samples
- Collect urine for urinalysis
- Calculate malaria clinical score if malaria counts are positive or at the discretion of the Principal Investigator or delegate.

If a volunteer still has unresolved symptoms or abnormal clinical laboratory results at the EOS, the Principal Investigator or delegate may ask them to return to the clinical site for appropriate follow-up until resolution.

6.2 PHARMACODYNAMIC PRIMARY ASSESSMENT

6.2.1 Malaria 18S qPCR

The presence and measurement of total malaria parasites in blood of volunteers participating in IBSM model will be determined by quantitative real-time polymerase chain reaction targeting the malarial 18S rRNA gene (referred to as malaria 18s qPCR). Malaria 18S qPCR is expressed as parasites/mL and will be used to determine primary endpoints including parasite reduction ratio over 48 hours (PRR_{48}), parasite clearance half-life ($Pt_{1/2}$), lag phase, number of volunteers whose parasitaemia fall below LOQ following administration of IMP, number of volunteers with recrudescence and time to recrudescence.

Malaria 18S qPCR will also be used to monitor the parasitaemia of volunteers in real time, in order to monitor safety and to decide if mandatory anti-malarial rescue treatment is required earlier than the scheduled Day 21±3.

As detailed in the Detailed Schedule of Activities Table 2 blood will be collected from volunteers to determine malaria 18S qPCR on Day -8 prior to inoculation with the malaria challenge agent, Day -4, then twice daily on Day -3, Day -2 and Day -1 at the discretion of the Principal Investigator or delegate. On Day 1, blood will be collected pre-IMP administration, then at the following hours post-IMP administration while confined in the clinical unit: 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 48, 60, 72, 84, 96 and 108 hours post-IMP administration. Time windows during confinement are as per Table 5.

Volunteers may then return morning and evening for malaria 18S qPCR sampling until parasite counts are <~500 parasites/mL or at the Investigator's discretion. Volunteers may then have daily visits for malaria 18S qPCR sampling at the Investigator's discretion. When parasite counts are <~200 parasites/mL, malaria 18S qPCR sampling may revert to alternate day visits at the Investigator's discretion. Once the malaria 18S qPCR results are negative, or low and stable, volunteers may be reviewed 3 times per week at the Investigator's discretion until mandatory treatment with rescue medication scheduled for Day 21±3.

In the case of anti-malarial rescue medication being required before Day 21±3, associated safety assessments including the Malaria Clinical Score must be continued as per the Detailed Schedule of Activities for rescue medication in Table 3 and must be performed at a minimum 72 hours after rescue treatment was completed.

After rescue medication has been commenced, a minimum of 2 negative 18S qPCR results are required prior to the EOS.

Further information may be found in the Laboratory Manual.

6.3 OTHER PHARMACODYNAMIC PARAMETERS

6.3.1 *pfs25* qRT-PCR

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) tests for the malarial gametocyte-specific mRNA transcript pfs25.

Bloods samples for qRT-PCR will be collected from Day 5 onwards if malaria 18S qPCR results suggest the presence of gametocytes in the volunteers' blood and/or at the discretion of the Principal Investigator or delegate.

6.3.2 Parasite Drug Resistance

Blood samples will be collected from volunteers if they experience recrudescence.

Parasite recrudescence is defined as ≥5,000 blood stage parasites/mL after initial parasite clearance accompanied by either a two-fold parasitaemia increase within 48 hours, or re-occurrence of malaria symptoms with a malaria clinical score ≥6.

Malarial parasites will be obtained from the samples and analysed to determine resistance to MMV533 and investigate molecular mechanisms responsible for resistance. Parasite drug resistance will be assessed by calculating the IC50 of IMP and determining the percentage of surviving parasites using *in vitro* drug sensitivity testing.

Samples may be shipped to a national or international reference laboratory for phenotypic assessment and parasite gene sequencing analysis. Further information on sampling may be found in the Laboratory Manual.

6.4 PHARMACOKINETICS

Blood sample handling for PK analysis is described in the Laboratory Manual. Exact time of PK sampling is to be respected and the actual time of sampling recorded in source document and into the eCRF.

Blood samples will be collected for PK analysis at the following timepoints:

- Day 1 pre-IMP administration;
- At the following hours post-IMP administration:
    - 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10.0, 12, 24, 48 72 and 96 hours;
    - Day 8 (168 hours), Day 14 (312 hours), Day 21 (480 hours) and EOS Day 28 (648 hours).

The permitted time windows for PK blood sampling are detailed in Table 5.

Table 5: Permitted PK and PD Blood Sampling Time Windows

Time point	Tolerance window
Pharmacokinetic/Pharmacodynamic Blood Samples	
In confinement	
Pre-dose	Within 120 min prior to IMP dosing
0.5-4 hours inclusive after IMP administration	± 15 min
6-12 hours inclusive after IMP administration	± 60 min
24-96 hours inclusive after IMP administration	± 120 min
Post-confinement Outpatient Visits	
Days 8, 14	± 2 days
Day 21, EOS	± 3 days

7 SAFETY ASSESSMENTS

- The schedules for all safety assessments/activities are summarised in the Schedule of Activities (Table 1) and in more detail in the Detailed Schedule of Activities (Table 2 and Table 3). Protocol waivers or exemptions are not permitted.
- All safety assessments may be conducted at unscheduled visits or timepoints if required for the volunteer's safety at the discretion of the Principal Investigator or delegate.
- When several assessments/activities are scheduled to take place at the same timepoint, the order is recommended to be: ECG, vital signs, blood sampling, IMP administration, meal (where applicable/appropriate), urine sampling.
- Exact timing for PK blood sampling is to be maintained – safety assessments/activities scheduled for these timepoints will be conducted ahead of the timepoint.

7.1 PHYSICAL EXAMINATION

Physical examination will include at a minimum: heart and respiratory auscultation; peripheral arterial pulse; pupil, knee, Achilles and plantar reflexes; peripheral lymph nodes and abdomen examination.

Symptom directed physical examination: body systems will be reviewed only if clinically indicated and at the discretion of the Principal Investigator or delegate. Symptom directed physical examination may be performed as required by the Principal Investigator or delegate throughout the study.

Full physical examination will be performed at Screening and at EOS.

After inoculation with the malaria challenge agent on Day -8, physical examination will also consider the following:

- Signs of malaria include fever (oral temperature above 38°C), chills/shivering/rigors, tachycardia, hypotension
- Symptoms of malaria include headache, myalgia, arthralgia, fatigue/lethargy, malaise, sweating/hot spells, anorexia, nausea, vomiting and abdominal discomfort.

Symptom-directed physical examination may be performed at the discretion of the Principal Investigator or delegate as outlined below:

- On Day -8 prior to inoculation with the malaria challenge agent,
- Once daily on Day -4, Day -3, and Day -2
- Upon admission to the clinical unit on Day -1 (PM),
- During confinement at the clinical unit from Day 1 until discharge, symptom-directed physical examination will be performed when clinically indicated including if signs or symptoms of malaria are identified.
- Prior to the discharge of volunteers from the clinical unit on Day 5 after confinement for IMP administration, clinical staff will check with the Principal Investigator or delegate to clarify if any volunteer will require a symptom directed physical examination.
- After discharge from the clinical unit on Day 5, symptom-directed physical examination will be performed at outpatient monitoring visits if clinically indicated, including if signs or symptoms of malaria are identified.

7.2 VITAL SIGNS, TEMPERATURE AND BODY MEASUREMENTS

7.2.1 Body Measurements and Temperature

Body weight (kg) and height (cm) will be measured at screening. Body weight (kg) will also be measured at the eligibility visit between Days -11 to -9 and at EOS.

Tympanic body temperature (°C) will be taken at screening, on Day -8 prior to inoculation with the malaria challenge agent, on Day -4, on Day -3 at least once and twice at the discretion of the Principal Investigator or delegate, twice daily on Days -2 and -1, then at the following times post-IMP administration during confinement: Day 1 pre-dose, then t = 1, 2, 4, 6, 8, 10, 12, 24, 30, 36, 48, 60, 72, 84, 96 and 108 hours post-IMP administration. After discharge from the clinical unit, oral body temperature will be taken at every subsequent outpatient visit to the clinical unit including EOS.

NOTE: On Day -8 after inoculation with the malaria challenge agent and prior to leaving the clinical unit, each volunteer will be issued a diary and thermometer to record their temperature in the event of symptoms of fever. The volunteers will bring their diaries to each subsequent visit to the clinical unit.

Time windows are as described for Vital Signs in Section 7.2.2.

7.2.2 Vital signs

- Screening and EOS: heart rate, systolic and diastolic blood pressure will be measured after 5 minutes in supine resting position, and after 3 minutes in standing position.
- For all other timepoints, heart rate, systolic and diastolic blood pressure will be measured after 5 minutes in supine resting position.

Vital signs will be measured at Screening, on Day -8 both prior to and approximately 60 minutes after inoculation with the malaria challenge agent, on Day -4, on Day -3 (at least once, and twice at the discretion of the Principal Investigator or delegate), twice daily on Days -2 and -1. From Day 1, vital signs will be measured pre-dose and at the following times post-IMP administration: 1, 2, 4, 6, 8, 10, 12, 24, 30, 36, 48, 60, 72, 84, 96 and 108 hours (prior to discharge from the clinical unit). After discharge from the clinical unit, vital signs will be measured at each outpatient visit (at least once per day if visits twice daily at the discretion of the Principal Investigator or delegate).

Permitted time windows for vital sign measurements during confinement:

- ± 10 min of PK sampling (see Table 5)
- ± 1h for timepoints where no PK sample is collected

For post-confinement visits, time windows are as per Table 5 when PK samples collected.

7.3 12-LEAD ELECTROCARDIOGRAM (ECG)

Standard 12-lead ECGs (safety ECGs) will be recorded after at least 10 minutes in supine position using a validated electrocardiographic device. The electrodes will be positioned at the same place for each ECG recording throughout the study (attachment sites of the leads will be marked with an indelible pen).

When triplicate ECGs are required, 3 ECGs will be recorded within 5 minutes with at least 1 minute between 2 replicates. Each ECG consists of a 10-second recording of the 12 leads simultaneously, leading to:

- A single 12-lead ECG (25 mm/s, 10mm/mV) printout with heart rate, PR, QRS, QT, QTc automatic correction evaluation (by the ECG device), including date, time, initials, and number of the volunteer, signature of the research physician, and at least 3 complexes for each lead. The Principal Investigator's or delegate's medical opinion on clinical significance and automatic values will be recorded in the eCRF. This printout will be retained at the site.

Triplicate ECGs will be conducted at the following timepoints:

- Screening visit
- Day -8 pre-inoculation with the malaria challenge agent
- Day 1 pre-dose

Single 12-lead safety ECGs will be conducted at the following timepoints:

- Day -1 morning, Day 1 at the following hours post-IMP administration:
  - 4, 6, 8, 12, 24, 48, 72 and 96
- Days 8 (±2 days) , 14 (±2 days), 21 (±3 days), pre-dose on day of rescue medication, 25 (±3 days) and EOS

Permitted time windows for 12-lead ECGs during confinement:

- ± 10 min of PK sampling, preferably before PK sampling (seeTable 5)
- ± 1h for timepoints where no PK sample is collected

For post-confinement visits, time windows are as per Table 5 when PK samples collected.

7.4 MALARIA CLINICAL SCORE

The Malaria Clinical Score will be used to quantify signs and symptoms of malaria in volunteers after they have been inoculated with the malaria challenge agent (see Appendix 2: Malaria Clinical Score).

The 14 signs/symptoms frequently associated with malaria are graded using a 4-point scale (Absent 0; mild: 1; moderate: 2; severe: 3) and summed to generate a total malaria clinical score (maximum score possible is 42).

To determine severity of the 14 signs/symptoms, the Principal Investigator or delegate will use the Common Terminology Criteria for Adverse Event Reporting (CTCAE) Grade

1 - 5 Version 5.0 Published: November 27, 2017. Mild (1) equates to CTCAE grade 1, Moderate (2) equates to CTCAE grade 2 and Severe (3) equates to CTCAE grade 3.

See Detailed Schedule of Activities Table 2 and Table 3 for the detailed schedule for recording the Malaria Clinical Score. The score will be recorded for volunteers prior to and approximately 60 minutes after inoculation with the malaria challenge agent on Day -8, once on Day -4, at least once, and twice at the discretion of the Principal Investigator or delegate on Day -3, and twice daily on Days -2 and -1. On Day 1, the Malaria Clinical Score will be recorded prior to IMP administration, and then at t = 2, 6, 12, 24, 30, 36, 48, 60, 72, 84, 96 and 108 hours post-IMP administration. After the volunteers are discharged from the clinical unit, the Malaria Clinical Score will be recorded at each visit when samples for Malaria 18S qPCR are taken, pre-dose on day of rescue medication and at EOS.

In the case of anti-malarial rescue treatment being required before Day 21±3, safety assessments including the Malaria Clinical Score must be continued as per the Detailed Schedule of Activities Table 2 and Table 3, and must be performed at a minimum 72 hours after rescue treatment was completed.

See Section 6.1.5 for Note on appropriate consideration of COVID-19 symptoms.

7.5 CLINICAL LABORATORY TESTS

Blood and urine will be collected for clinical laboratory safety tests conducted at the clinical unit's preferred Vendor laboratory unless otherwise stated. Handling of samples is described in the Pathology Vendor Laboratory Manual.

- For the biochemistry and haematology safety laboratory tests scheduled for Screening, safety visit during Days -11 to -9, and on Day -1, volunteers must attend the clinical unit having fasted for at least 8 hours. Other scheduled timepoints for safety laboratory testing do not require the volunteer to have fasted overnight.
- The Principal Investigator or delegate must review all results and assign clinical significance to any out of range results. Results must also be reviewed with reference to Section 4.3.8 and Adverse Events of Special Interest (AESIs) as defined in Section 7.9.5.
- Results of safety laboratory tests must be available and reviewed by the Principal Investigator or delegate prior to inoculation with the malaria challenge agent and IMP administration (except results of Day 1 pre-dose safety sampling). On Day -1, the blood sample for haematology and biochemistry clinical laboratory safety tests will be collected approximately 24 hours prior to IMP dosing to check for potential safety signals occurring after inoculation with the malaria challenge agent. Results must be reviewed by the Investigator prior to confirming IMP dosing may proceed.

7.5.1 Safety Blood Tests

Blood for safety blood tests will be collected:

Screening (fasted), once between Day -11 to Day -9 (fasted, with results to be available prior to inoculation with the malaria challenge agent on Day -8), Day -3 (LFTs only), Day -1 (fasted; NOTE: results must be available for review by the Principal Investigator or delegate prior to IMP administration on Day 1 to check for safety signals resulting from inoculation with the malaria challenge agent), Day 1 pre-dose and at the following hours post-IMP administration: 24, 48, 72 and 96 hours prior to discharge from the clinical unit.

After discharge from the clinical unit, blood will be collected on Days 8 (±2 days), 14 (±2 days), 21 (±2 days) (pre-dose on day of rescue medication), Day 25 (±2 days) and EOS.

7.5.1.1 Haematology

Red blood cell count, haematocrit, haemoglobin, white blood cell count with differential count (neutrophils, eosinophils, basophils, monocytes, and lymphocytes), platelets.

7.5.1.2 Biochemistry

- Plasma/serum electrolytes (sodium, potassium, chloride, calcium, magnesium);
- Liver function tests (AST, ALT, alkaline phosphatase, gamma-glutamyl transferase, glutamate dehydrogenase, total and conjugated bilirubin, biliary acids);
- Renal function (urea, creatinine)
- Metabolism (glucose, albumin, total proteins, total cholesterol, triglycerides)
- Potential muscle toxicity (creatinine phosphokinase)
- CRP
- Screening visit only: glucose-6-phosphatase (G6PD)

7.5.2 Coagulation

Blood will be collected for coagulation studies only at screening and will include international normalized ratio (INR) and activated partial thromboplastin time (APTT).

7.5.3 Serology

Blood will be collected for serology tests at screening and EOS and will include: hepatitis B surface antigen (HB sAG), anti-hepatitis B core antibodies (anti-HBc Ab), hepatitis C antibodies, anti-HIV1 and anti-HIV2 antibodies. Red blood cell alloantibodies will also be assayed for all volunteers.

7.5.4 Pregnancy and Follicle Stimulating Hormone (FSH) Testing

7.5.4.1 Pregnancy Testing

Blood will be collected from all female volunteers for serum β-human chorionic gonadotropin (β-hCG) testing at screening for the study. Blood will be collected from WOCBP for serum β-hCG testing at EOS for the study.

Urine will be collected from volunteer WOCBP for β-hCG testing by the clinical unit: for visit(s) between Days -9 to -11 prior to inoculation with the malaria challenge agent and Day -1 prior to IMP administration.

Blood will also be collected from a female volunteer for unscheduled serum β-hCG testing if their urine β-hCG test returns a positive result.

7.5.4.2 Confirming Menopause

Blood will be collected from post-menopausal female volunteers for follicle stimulating hormone (FSH) testing at screening only.

7.5.5 Red Blood Cell alloantibodies

Blood will be collected from volunteers only at Screening and EOS to test for red blood cell alloantibodies.

7.5.6 Urinalysis

Urine will be collected as part of the clinical laboratory safety testing at the same timepoints as for safety blood tests above in Section 7.5.1 up to and including t = 24 hours

post-dose (except Day -3), and **then only** at t = 72 hours post-dose, Days 8 (±2 days), 14 (±2 days), 21 (±2 days) (pre-dose on day of rescue medication) and EOS. The urinalysis will test for proteins, glucose, erythrocytes, leucocytes, ketone bodies and pH.

Qualitative: a dipstick test will be performed on a freshly voided urine sample.

Quantitative: if the urine sample dipstick test is positive for glucose, protein, erythrocytes or leucocytes, quantitative measurement of these parameters by pathology laboratory is required.

7.6 RETENTION SAMPLES

IMP SAFETY SERUM SAMPLES

A 5mL blood sample will be collected on Day -8 prior to inoculation with the malaria challenge agent and at EOS.

Volunteers consent to this mandatory collection and storage and the use of the sample for safety assessments when they sign the Informed Consent Form for the study. These samples will be used if any unexpected safety issue related to the IMP occurs to ensure that a pre-administration baseline value is available for parameters not previously planned/assessed (eg, serology). These samples will be stored at Nucleus Network. If the sample is not used, the Principal Investigator or delegate will destroy it at the end of the study and final CSR is available after Sponsor approval.

INOCULUM SAFETY SERUM SAMPLES

A 5mL blood sample will be collected on Day -8 prior to inoculation with the malaria challenge agent and at EOS.

Volunteers consent to this mandatory collection and storage and the use of the sample for safety assessments related to the inoculum when they sign the Informed Consent Form for the study. Inoculum safety samples will be retained for at least 15 years from the completion of the study at the QIMR Berghofer Institute.

RESEARCH SERUM SAMPLES

Under a separate protocol and consenting process, research serum samples may be collected for storage and use in future malaria research. The separate study will be under the local sponsorship of QIMR Berghofer Institute and will involve a protocol and consent document approved by the QIMR HREC.

Only if the volunteer consents to the separate study, up to four (4) blood samples will be collected at timepoints that coincide with visits under the MMV533-VIS protocol including timepoints prior to and after inoculation (see SOAs Table 1, Table 2 and Table 3 in Section 1.3).

Blood volume of each sample may be up to and not exceeding 30 mL:

- And will be an appropriate volume for the day/timepoint, AND
- The total volume of blood taken including the main study samples will not exceed 450 mL per month.

A volunteer's decision can be changed at any time prior to the EOS by notifying QIMR, as local Sponsor, in writing. However, if a volunteer consents to future use and some of their blood has already been used for research purposes, the information from that research may still be used.

7.7 ALCOHOL AND DRUG SCREENING

Alcohol will be measured by breath test at the clinical unit.

Urine will be collected for drug screening, using suitable dipstick test at the clinical unit for: amphetamines, methamphetamines, barbiturates, benzodiazepines, cocaine, methadone, opiates, phencyclidine, tetrahydrocannabinol, tricyclic antidepressants.

Alcohol and drug screening will be conducted at screening, Day -8 prior to inoculation with the malaria challenge agent and Day -1 prior to IMP dosing.

Unscheduled alcohol and drug screening may be performed if required for volunteer safety or to assess continued eligibility at the discretion of the Principal Investigator or delegate.

7.8 BECK DEPRESSION INVENTORY

Originally described by Beck et al (1961), the Beck Depression Inventory (BDI) is a validated objective tool for the assessment of depression. Updated in 1996, the BDI-II is a 21-item, self-report rating inventory that measures characteristic attitudes and symptoms of depression, and volunteers will be required to complete the BDI-II at Screening for eligibility.[12] The BDI-II takes approximately 10 minutes to complete, although clients require a fifth – sixth grade reading level to adequately understand the questions. A score of ≥20 at screening and/or a response of 1, 2 or 3 for item 9 indicating current suicidal ideation is exclusionary. A BDI-II score of 17 to 19 may be enrolled at the discretion of the Principal Investigator if they do not have a history of the psychiatric conditions mentioned in exclusion criterion 26 and their mental state is not considered to pose additional risk to the health of the volunteer or the execution of the study and interpretation of the data gathered. The results will be entered into the eCRF.

7.9 ADVERSE EVENTS (AEs)

The Principal Investigator or delegate and clinical unit staff are responsible for detecting recording and reporting events that meet the criteria and definition of adverse events as described below. Adverse events may be reported by the volunteer (including in the diary) or observed by the Principal Investigator, delegate or other clinical site staff.

All AEs will be followed until the event has resolved, no further medically relevant information from the event can be expected and it is acceptable to discontinue follow-up of the event in the assessment of the Principal Investigator. The Principal Investigator should continue to follow up AEs that were unresolved at the volunteer's EOS as long as medically required or until the volunteer has been satisfactorily referred to a general practitioner or medical specialist as appropriate.

Medically untoward events occurring in volunteers between the time of consent (screening) and the time of inoculation with the malaria challenge agent will be considered medical history and not recorded as AEs.

See Section 6.1.5 for Note on appropriate consideration of COVID-19 symptoms.

7.9.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a clinical trial volunteer administered a medicinal product, and that does not necessarily have a causal relationship with this treatment. In this study, AEs will also be recorded for the period after inoculation with the malaria challenge agent and prior to IMP administration.

AEs include, but are not limited to:

- A new symptom, sign or medical condition which develops after inoculation with the malaria challenge agent.
- A disease or medical condition detected or diagnosed during the course of the study even though it may have been present prior to the start of the study.
- An exacerbation of a pre-existing medical condition/disease.
- An increase in frequency or intensity of a pre-existing episodic disease or medical condition.
- Continuous persistent disease or symptoms present at study start that worsen following the start of the study.
- An abnormal assessment (e.g., change on physical examination, ECG finding) if it represents a clinically significant finding that was not present at study start or worsened during the course of the study.
- An abnormal laboratory test result if it represents a clinically significant finding (e.g., CTCAE grade 2 or above), symptomatic or not, which was not present at study start or worsened during the course of the study or led to dose reduction, interruption or permanent discontinuation of study treatment.

Abnormal laboratory findings and other objective assessments should NOT be routinely captured and reported as AEs. However, abnormal laboratory findings or other objective measurements that meet the following criteria should be captured and reported in the AE Section of the eCRF:

- The result meets the criteria for reporting as an SAE (Section 7.10.1) or AESI (Section 7.9.5);
- The test result is associated with accompanying symptoms, and/or
- It requires additional diagnostic testing or medical/surgical intervention, and/or
- It leads to a change in IMP dosing, or discontinuation from the study, significant additional concomitant drug treatment, or other therapy, and/or
- It is considered by the Principal Investigator (or delegate) or Sponsor to be clinically significant or represent a clinically significant change from baseline.

AEs will not include:

- A medical or surgical procedure such as surgery, endoscopy, tooth extraction, or transfusion (although the condition that leads to the procedure may be an AE).
- A pre-existing disease or condition present at the start of the study that does not worsen during the study.
- Any situation where an untoward medical occurrence has not occurred (for example, hospitalizations for cosmetic elective surgery or social admissions).

7.9.2 Causal Relationship to Investigational Medicinal Product and Other Study Treatments

The Principal Investigator or delegate is required to assess the causality to study interventions for all AEs, and must indicate this in the source document and eCRF (study interventions include: inoculation with the malaria challenge agent, IMP administration and administration of anti-malarial rescue medication).

An adverse event with reasonable causal relationship to the IMP means there is evidence or argument to suggest a causal relationship.

If considered related:

- The temporal relationship between the event and the administration of the IMP is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the volunteer's medical condition, other therapies or accident.

If considered not related:

- The event can be readily explained by other factors such as the volunteer's underlying medical condition, concomitant therapy or accident and no plausible temporal or biologic relationship exists between the IMP and the event.

7.9.3 Severity Grading of Adverse Events

The severity of AEs will be recorded in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) v5.0, published 27 November 2017. This guidance provides a common language to describe levels of severity, to analyse and interpret data, to scale the aggregate AE score, and to articulate the clinical significance of all AEs.

The severity of AEs will be graded as follows:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care activities of daily living.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

An AE that is assessed as severe is not the same as a serious AE. Severity is a category utilized for rating the intensity of an event and both AEs and SAEs can be assessed as severe. An AE is defined as 'serious' when it meets one of the pre-defined serious outcomes as described below in Section 7.10.1.

7.9.4 Documentation of Adverse Events

The Principal Investigator or delegate is responsible for reviewing all documentation related to each AE (including volunteer diaries), and for recording all relevant AE/SAE information in source document and in the eCRF. The Principal Investigator or delegate will attempt to establish a diagnosis of the AE based on signs, symptoms and/or other clinical information, and where possible the diagnosis will be documented as the AE/SAE (and not individual signs or symptoms). Clinically significant abnormal safety assessment findings should be documented as AEs unless regarded as signs or symptoms of an AE.

The following information should be recorded for all AEs:

- Description
- Dates and times of onset and resolution
- Time of onset relative to IMP administration, inoculation with the malaria challenge agent, and/or administration of antimalarial rescue medications
- Seriousness
- Severity
    - In the source data, the description of the AE will report the various severities observed over time. If the severity of an AE increases, separate

AEs per severity grading will be recorded into the eCRF.If the AE resolves and then reoccurs, then two AEs will be reported.

- Action taken in response to the AE regarding IMP:
  - No action taken, or
  - Rescue medication administered instead of IMP, or
  - Not applicable.
- Outcome of AE:
  - Recovered/resolved, or
  - Recovering/resolving, or
  - Not recovered/not resolved, or
  - Recovered with sequelae/resolved with sequelae
  - Fatal
  - Unknown
- Relationship to the IMP or procedures conducted during the trial including inoculation with the malaria challenge agent or treatment with rescue medication (causality assessment).

7.9.5 Adverse Events of Special Interest (AESI)

Adverse Events of Special Interest (AESIs) may be serious or non-serious, and are defined by the Sponsor as being of specific scientific and/or medical concern to the Sponsor's product or programme. AESIs will be required to be monitored and reported promptly to the Sponsor by the Principal Investigator or delegate. Such an event may require further investigation in order to characterise and understand it.

All AESIs (both serious and non-serious) must be notified to the pharmacovigilance provider (Prime Vigilance) within 24 hours of the clinical study staff becoming aware of the event (see Section 7.10.3 below). Reporting should be via a Serious Adverse Event Report Form marked as 'AE of special interest'. Follow-up information will be submitted in a timely fashion as it becomes available.

7.9.5.1 Hepatic AEs of Special Interest

- Any ALT or AST above 5×ULN,
- An elevation in bilirubin 2×ULN,
- Any AST or ALT above 2×ULN and Total Bilirubin Level (TBL) >1.5×ULN or INR >1.4,
- Any AST or ALT above 2×ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (eosinophil percent or count above the ULN).

7.9.5.2 Cardiac AEs of Special Interest

- QTcB or QTcF at any time >480 msec,
- Bundle branch block (except right bundle branch block that was present prior to IMP administration),
- Any arrhythmia, except:
  - Sinus bradycardia that is clinically asymptomatic, and not associated with any other relevant ECG abnormalities,
  - sinus tachycardia that is clinically asymptomatic, and associated with a body temperature >38.0°C, and not associated with any other relevant ECG abnormalities,
  - Respiratory sinus arrhythmia,
  - Wandering atrial pacemaker,

- Isolated, single premature atrial/ventricular complex (i.e., no bigeminy, trigeminy, couplets, triplets or salvos) that does not occur more than once in a particular ECG tracing.

7.9.5.3 Haematological AEs of Special Interest

- Haemoglobin drop >20.0 g/L from baseline prior to inoculation,
- Absolute neutrophil count $<0.5 \times 10^9$/L,
- Platelet count $<75 \times 10^9$/L.

7.9.5.4 Dermatological AEs of Special Interest

Clinical signs of possible cutaneous adverse reactions such as:

- Dermatitis,
- Rash, including erythematous, macular, papular, maculopapular, pruritic, pustular, and vesicular.

If one of these cutaneous reactions are observed and when feasible, pictures of the lesions should be obtained.

Dermatological AEs do not need to be reported as AESIs if clearly unrelated to inoculum or study drug (e.g. rash from cannula dressing or ECG dots).

7.9.6 Treatment of Overdose

Overdose of IMP is considered unlikely as it is a single dose administered under supervision at the clinical unit. However symptomatic overdose of IMP or of other study treatments/interventions (ie, malaria challenge agent, malaria rescue medications) is an event suspected by the Principal Investigator or delegate, or notified by the volunteer, and defined as at least twice the intended dose within the intended therapeutic interval adjusted according to the tested drug. Such an event should be reported promptly to the Sponsor as for AESIs. Asymptomatic overdose will be reported as a standard AE.

7.10 SERIOUS ADVERSE EVENTS (SAES)

7.10.1 Definitions of Serious Adverse Events (SAEs)

A serious adverse event (SAE) is any AE that:

- Results in death, or
- Is life-threatening, and/or
- Requires inpatient hospitalisation or prolongs existing hospitalisation, and/or
- Results in persistent or significant disability or incapacity, and/or
- Is a congenital anomaly or birth defect, and/or
- Constitutes a possible Hy's Law case:
    - Hy's Law case is defined as a volunteer with any value of alanine or aspartate aminotransferase greater than 3×ULN together with an increase in total bilirubin to a value greater than 2×ULN and not associated to an alkaline phosphatase value greater than 2×ULN (FDA Guidance on Drug Induced Liver Injury: Premarketing Clinical Evaluation [2009]).

Note: Life-threatening in the definition of an SAE refers to an event in which the volunteer was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.

Note: Medical and scientific judgement should be exercised in deciding whether an AE should be classified as serious in other situations. Important medical events that are not immediately life-threatening or do not result in death or hospitalization, but may

jeopardise the volunteer or may require intervention to prevent one of the other outcomes above should also be considered serious.

Planned procedures that require admission to hospital for this study, or any planned elective procedure that requires hospitalization are not considered SAEs unless the underlying condition has worsened or the volunteer's condition worsens after the procedure.

7.10.2 Pregnancy

Pregnancy itself is not defined as an AE/SAE. Any complication or termination of pregnancy for medical reasons are to be reported as an AE/SAE. Spontaneous abortion, still birth or congenital anomaly must be reported as an SAE.

Any WOCBP (Woman of Child-Bearing Potential) enrolled in the study who becomes pregnant during the study and the following 60 days after the dosing should be followed through delivery or termination of the pregnancy. The Investigator will collect pregnancy information and report to the Sponsor within 24 hours of becoming aware of a volunteer's pregnancy. Follow-up will generally not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Once pregnancy is confirmed, pregnant female volunteers will be immediately withdrawn from the study as outlined in Section 4.3.5.

Where possible, the Investigator will also attempt to collect and report information regarding pregnancy outcomes of female partners of any male volunteers who were administered IMP in this study and the following 60 days after the last dosing. Appropriate signed informed consent will be required directly from the pregnant female partner to obtain and report this information. Any volunteer's female partner who becomes pregnant during the study should be followed through delivery or termination of the pregnancy.

7.10.3 Reporting for Serious Adverse Events (24 hours)

If any SAE occurs, the Investigator will take immediate appropriate action and strive to identify the causes of the event/s. The Investigator must notify the SAE to the pharmacovigilance provider (Prime Vigilance) by email within 24 hours of becoming aware of the event. Safety reporting for the study will be further outlined in the study-specific Safety Plan.

All reports must be signed by the Principal Investigator or delegate and notified to Prime Vigilance preferably by email or fax to:

Email: MMV@primevigilance.com

Back-up fax number: +44 800 471 5694

Prime Vigilance Contact:
Andreja Baricevic
e-mail: andreja.baricevic@primevigilance.com
Phone: +44 385 1 46 28 183
Mobile: +44 385 99 2680 787
Head Office: +44 1483 307920

Any copies of volunteer's medical records provided for SAE reporting must have all volunteer identifiers redacted before submission.

The SAE report form will always be completed as thoroughly as possible with all available details of the event and signed by the Principal Investigator or delegate. If the Principal Investigator or delegate does not have all information regarding an SAE, he/she

will not wait to receive additional information before reporting the event. A follow-up SAE report should be completed within 14 days, or if there is no new information the SAE report form should be updated when additional information is received.

The Principal Investigator or delegate will always provide an assessment of causality at the time of the initial report.

Email transmission of the SAE report form is the preferred method to transmit this information to Prime Vigilance. In rare circumstances notification by telephone is acceptable, with a copy of the SAE report form sent by overnight mail.

The Principal Investigator or delegate, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the HREC.

7.11 SAFETY REVIEW COMMITTEE (SRC)

The SRC will be responsible for decisions related to the safety of volunteers and to inform decision making on trial progression and details will be outlined in the Safety Plan. The required data to be reviewed will be detailed in a separate trial-specific Safety Review Committee Charter and will be sent to the SRC prior to all scheduled and any ad hoc SRC meetings associated with the trial.

The Safety Review Committee (SRC) will be composed at a minimum of the Sponsor's Medical Director, the Principal Investigator, the study Medical Monitor and the Malaria Physician. Representative/s for PK analysis will also be included. The study will proceed cohort by cohort with SRC review in between each cohort. The SRC may decide to discontinue the study after any of the cohort reviews.

The SRC will choose the dose levels for cohorts 2 and 3 based upon their review of the appropriate data from cohort 1. For each of the cohorts, the SRC will review safety and tolerability data up to Day 22 and PK and PD analysis of data up to day 15 before the next cohort is given permission to proceed with inoculation with the malaria challenge agent on Day -8.

8 STATISTICAL ANALYSES

8.1 GENERAL APPROACH

The following sections describe the statistical analysis as it is foreseen during the planning phase of trial. A detailed Statistical Analysis Plan (SAP) will be finalised and approved prior to database lock and will provide details of all analyses to be performed as well as the format of listings and tables to be provided for completion of the clinical study report (CSR). Any deviations from the SAP will be described and justified in the final CSR. The PK and PD analyses may be detailed in a separate analysis plan, as deemed appropriate.

The general analytical approach for all safety endpoints will be descriptive in nature. Unless otherwise stated, the following statistical approaches will be taken:

Continuous variables: Descriptive statistics will include the number of non-missing values, mean, standard deviation (SD), median, minimum, and maximum. The minimum and maximum values will be presented to the same number of decimal

	places as recorded in the CRF; mean, median and SD will be presented to one more decimal place than the raw data.
Categorical variables:	Descriptive statistics will include frequency counts and percentages per category. Percentages will be rounded to one decimal place, with the denominator being the number of volunteers in the relevant population with non-missing data.
Imputation:	No missing data will be imputed.
Confidence intervals (CIs):	If required, CIs will be two sided and will use 95% confidence levels. Any analyses requiring significance testing will use a two-sided test at the 5% significance level.
Baseline:	The inoculation baseline will be defined as the last available assessment prior to the inoculation.
Unscheduled assessments:	Unscheduled visits will be excluded from summary tables.
Early termination visits:	Assessments conducted at Early Termination will be excluded from summary tables.

8.2 SAMPLE SIZE AND JUSTIFICATION

Up to 24 volunteers (8 volunteers per cohort, all receiving MMV533). The sample size of the study is based on experience from previous Phase 1 IBSM trials.

8.3 ANALYSIS SETS AND SUB-SETS

8.3.1 Analysis Sets

In the first instance, two (2) analysis datasets will be used for study analyses: Full Analysis Set (FAS) and Safety Set.

Additional analysis populations may be defined in the SAP or the PK Analysis Plan.

The number of volunteers in each analysis set will be summarised, with a corresponding listing.

8.3.1.1 Full Analysis Set

The Full Analysis Set (FAS) will consist of all enrolled volunteers. The FAS will be used to assess all volunteer disposition, baseline, demographic and protocol deviation data.

8.3.1.2 Safety Set

The Safety Set will include all enrolled volunteers who received at least one dose (full or partial) of IMP.

8.4 VOLUNTEER DISPOSITION

A listing of volunteer disposition will present volunteer dates of informed consent, enrolment, randomisation, key visits as well as study completion details. Early termination data, including the reason for early termination will be listed in an additional listing.

A volunteer disposition summary table will present, at a minimum, the number of volunteers who completed the study per protocol and the number of volunteers who discontinued classified by reason for early termination.

8.5 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

8.5.1 Demographics

Demographic data will be listed for all enrolled volunteers and summarised by treatment arm and overall.

8.5.2 Medical History

All medical history data will be listed, grouped by volunteer.

Medical history will be coded using the Medical Dictionary for Regulatory Activities and summarised by system organ class (SOC) and preferred term (PT).

8.5.3 Prior Medications

Prior medications will be listed for all enrolled volunteers. Prior medications are defined as any medication that is started before first inoculation with the malaria challenge agent, regardless of when it ended. If a medication has a missing or partial missing start/end date or time and it cannot be determined whether it was taken before IMP or concomitantly, it will be considered as prior and concomitant.

8.5.4 Eligibility

Eligibility will be listed for all enrolled volunteers.

8.6 PROTOCOL DEVIATIONS

All protocol deviations will be listed. A protocol deviation summary table will present the total number of protocol deviations as well as the number of volunteers who reported at least one protocol deviation, broken down by deviation type.

8.7 TREATMENT EXPOSURE

Volunteer exposure to all protocol-specified treatments will be listed and summarised.

8.8 SAFETY (PRIMARY ENDPOINT)

8.8.1 Safety Endpoints

Safety and tolerability will be assessed by clinical review of the following parameters:

- AEs (including SAEs and AESIs)
- Vital signs
- 12-lead ECG
- Haematology, chemistry, urinalysis
- Physical examination

All descriptive statistics for safety parameters will be evaluated using the Safety Set.

8.8.2 Adverse Events

All AE data will be listed for each volunteer, including severity, relationship to IMP, relationship to non-IMP protocol-specific treatments, outcome and actions taken. In addition, listings of AEs leading to discontinuation of the study, SAEs and deaths, will be provided as applicable.

All AE summaries will be restricted to Treatment Emergent Adverse Events (TEAEs) only, where a TEAE is defined as an AE that commences on, or after, the first administration of IMP up to the end-of-study (EOS) visit (inclusive). TEAEs without an onset date or time will be defined as treatment emergent except if an incomplete date (e.g., month and year) clearly indicates that the event started prior to first administration of IMP, or if the AE stop date indicates that the event started and/or stopped prior to the first administration of IMP.

The safety analysis will also be done specifically for the inoculation period, defined as the time from the inoculum injection (Day-8) to the day before the IMP administration (Day-1) visit (included).

Adverse events (AE) will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA) and grouped by system organ class (SOC) and preferred terms (PT). Their severity will be graded according NCI-CTCAE v5.

At a minimum, the following AE summary tables will be provided:

- Overall summary of TEAEs
- All TEAEs
- TEAEs by severity
- TEAEs by relationship
- Serious TEAEs
- TEAEs leading to study withdrawal

8.8.3 Concomitant Medications

Medications used in this study will be coded using WHODrug Global.

Concomitant medications are defined as medications continued or newly received at or after administration of IMP, through to the End of Study visit.

If a medication has a missing or partial missing start/end date or time and it cannot be determined whether it was taken before initial treatment or concomitantly, it will be considered as prior and concomitant.

Concomitant medications will be summarised by Anatomical Therapeutic Class (ATC) and preferred name (PN). The summary tables will show the number and percentage of volunteers taking each medication by ATC and PN.

For the summaries of prior and concomitant medications, volunteers who take the same medication (in terms of the ATC and PN) more than once will only be counted once for that medication.

8.8.4 Laboratory Findings

Laboratory parameters will be listed by volunteer and visit, including:

- haematology,
- biochemistry,
- coagulation,
- urinalysis,
- serology,
- red blood cell antibodies
- G6PD status (screening only)
- drug screening,

- alcohol screening
- FSH, and
- pregnancy test.

Haematology, biochemistry and continuous urinalysis laboratory data will be summarised for each scheduled visit, including observed values, absolute change from each baseline. Categorical urinalysis results will be summarised for each scheduled visit using frequency tabulations.

Any available microscopic urinalysis will be listed.

8.8.5 Physical Examination

Physical examination parameters will be listed for all volunteers and visits.

8.8.6 Body Measurements and Body Temperature

Height and weight will be listed for all volunteers and visits.

Observed values (including changes from each baseline) will be summarised for weight.

Body temperature will be listed for all volunteers and visits.

Observed values, as well as absolute changes from each baseline, will be summarised descriptively for all vital sign parameters by visit.

8.8.7 Vital Signs

Vital sign parameters will be listed for all volunteers and visits.

Observed values, as well as absolute changes from each baseline, will be summarised descriptively for all vital sign parameters by visit.

8.8.8 12-lead ECG

ECG parameters will be listed for all volunteers and visits. Triplicate ECGs will be presented in listings for individual readings as well as the mean for each triplicate. The triplicate means will be used for the summary table.

Observed values, as well as absolute changes from each baseline, will be summarised descriptively for all ECG parameters by visit. A categorical analysis of QTcF and/or QTcB changes from each baseline may also be presented.

8.9 PHARMACOKINETIC ENDPOINTS

8.9.1 Pharmacokinetic Parameters

MMV533 PK profiles will be plotted individually and summarized by dose group.

MMV533 PK parameters will be calculated using non-compartimental methods from plasma concentration-time data and will be summarized by dose group using descriptive statistics.

8.9.2 Pharmacokinetics/pharmacodynamics

A population PKPD model will be derived from the plasma concentrations of MMV533 and asexual parasitemia levels deduced from the total parasitemia measured by qPCR 18s and female gametocytemia measured by RT-PCR pfs25 in the challenge volunteers. It is achieved by sequentially developing a population PK model to describe the observed individual PK profiles and a population PD model for quantifying the relationship of plasma concentrations and the parasite killing/clearance. The effect MMV533 concentration on parasite killing/clearance will be described with an Emax model.

Alternative models may be tested if the exploration of the data indicate a different behavior, e.g. if a lag time is observed before the parasites are cleared. In such case, the models tested will be assessed by goodness-of-fit plots and BIC values to retain the best model. The estimated population PKPD parameters and between-subject variability as well as their relative standard errors will be provided. Other key parameters will be derived from the PKPD model including the minimum inhibitory concentration (MIC), the minimal parasiticidal concentration (MPC90), and the parasite reduction rate in 48 h (PRR48).

8.10 PHARMACODYNAMIC ENDPOINTS

Individual total parasitemia (measured by qPCR 18s) and female gametocytemia (measured by RT-PCR pfs25) profiles will be plotted. The individual asexual parasitemia profiles will be calculated as follows: if the gametocytemia level is lower than 10% of the total parasitemia level, the asexual parasitemia level is assumed to be equal to the total parasitemia level; otherwise, the asexual parasitemia is considered unknown. The asexual parasitemia profiles will be plotted individually and summarized by doses (median +/- 95% CI).

The pharmacodynamic parameters (PRR, Pt1/2, lag phase) calculated as described in Marquart et al[13] will be summarized using descriptive statistics (n, mean, geometric mean, standard deviation, median, Q1, Q3, minimum, and maximum) by dose group. The number and percentage of volunteers with absence of asexual parasitemia, the number and percentage of volunteers with recrudescence and the time to recrudescence will be summarized by dose group.

8.11 EXPLORATORY PARAMETERS

If applicable, exploratory parameters will be summarized by treatment group using descriptive statistics and may be reported separately.

8.12 INTERIM ANALYSIS

This trial has no formal interim analysis.

9 STUDY ADMINISTRATION

9.1 ETHICAL CONSIDERATIONS

9.1.1 Ethical Principles

This clinical study was designed and shall be implemented and reported in accordance with the Declaration of Helsinki, the Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2) dated 09 November 2016 and the National Statement on Ethical Conduct in Human Research, (2007 – updated 2018).

9.1.2 Informed Consent

Eligible volunteers may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC/HREC-approved informed consent. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be as per local regulatory requirements and documented in the volunteer source documents. A copy of the signed patient information and consent form will be provided to the volunteer.

9.1.3 Investigator and Human Research Ethics Committee (HREC) Responsibilities

Before initiating a trial, the investigator/institution should obtain approval/favorable opinion from the Human Research Ethics Committee (HREC) for the trial protocol, written informed consent form, consent form updates, volunteer recruitment procedures (e.g., advertisements) and any other written information to be provided to volunteers.

Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to the Sponsor appointed monitors, auditors, Quality Assurance representatives, HREC representatives, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform the Sponsor immediately that this request has been made.

9.2 PROTOCOL ADHERENCE

This protocol defines the study objectives, the study procedures and the data to be collected on study volunteers. Additional assessments required to ensure safety of volunteers should be administered as deemed necessary on a case by case basis. In this instance the Sponsor medical monitor must be advised before or as soon as possible after such assessments are conducted.

Under no circumstances should an investigator collect additional data or conduct any additional procedures for any research related purpose involving any investigational drugs. Investigators ascertain they will apply due diligence to avoid protocol deviations. If an investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by the Sponsor and approved by the HREC it cannot be implemented. All protocol deviations will be recorded and then reported in the clinical study report (CSR), including but not limited to the following deviation categories:

- Informed consent
- Eligibility
- Visit not done
- Visit performed out of window
- Study procedure not done
- Study procedure done out of window
- Safety reporting
- Investigational Product
- Privacy and Data Protection
- Concomitant Medication
- Other (including disruptions to the trial due to COVID 19 that are not per protocol)

9.3 PROTOCOL AMENDMENTS

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the Sponsor and the HREC prior to implementation. Only amendments that are intended to eliminate an apparent immediate hazard to volunteers may be implemented immediately provided the Sponsors medical monitor is notified as soon as possible after the event and the reviewing HREC is subsequently notified.

Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any volunteer included in this study, even if this action represents a deviation from the protocol. In such cases, the Medical Monitor and Sponsor must be notified immediately.

9.4 DATA HANDLING AND RECORD KEEPING

Volunteers will be assigned a unique identifier when participating in the study, and any volunteer datasets or records that are transferred to the Sponsor or CRO will only contain this identifier. Any other identifiable information about the volunteer will not be transferred. The Principal Investigator will ensure procedures are in place to appropriately protect the confidentiality of the volunteer records and data, including adequate safe guards for digital/computer access. The volunteers will be informed that their personal study-related data will be used by the Sponsor and that their medical records may be examined by auditors and regulatory agencies.

Study-related volunteer data will be entered into electronic case report forms (CRFs) by personnel authorized by the Principal Investigator, except for data that may be transmitted to the Sponsor or CRO electronically (such as laboratory data). The Principal Investigator is responsible for ensuring that accurate source documents for all data entered into the CRF are maintained at the study site and that the eCRF is completed accurately. Guidelines for eCRF completion including correcting data and responding to data queries will be provided by the Sponsor or CRO.

All study documents including source documents and signed PICFs must be retained by the Principal Investigator for at least 15 years and according to local regulatory requirements. No study documents may be destroyed or transferred during the retention period without the Sponsor being directly notified in writing.

9.5 QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor or CRO maintains responsibility for quality assurance of the data and for data management, and retains accountability for actions delegated to other parties (including CRO). Study monitors appointed by the Sponsor or CRO will conduct ongoing visits to the study sites to confirm the CRF data is accurate according to source documents and complete, and that the study is being appropriately conducted according to the protocol, ICH GCP and local regulatory requirements. Monitoring visits may be conducted remotely if required due to Corona virus disease 2019 (COVID 19) safety reasons.

9.6 PUBLICATION POLICY

Neither the complete nor partial results of the study achieved under this protocol, nor any of the information provided by the Sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study Sponsor. Any Investigator involved with this study is obligated to provide the Sponsor with complete study results and all data derived from the study.

Results of this trial will be either submitted for publication and/or posted in a publicly accessible database of clinical trial results.

10 REFERENCES

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11 APPENDICES

APPENDIX 1: SPONSOR APPROVED CLINICALLY ACCEPTABLE INCLUSION LABORATORY RANGES

Test	Unit	Acceptable Inclusion Range	
		Low	High
Sodium	mmol/L	130	150
Potassium	mmol/L	3.0	5.5
Chloride	mmol/L	0.90 x LLN	1.10 x ULN
Calcium (Corrected)	mg/dL	0.90 x LLN	1.10 x ULN
Phosphate	mmol/l	0.90 x LLN	1.10 x ULN
Bicarbonate	mmol/l	0.90 x LLN	1.10 x ULN
Glucose Fasted	mg/dL	N/A	1.0 x ULN
Urea	mg/dL	N/A	1.75 x ULN
Uric acid	mg/dL	N/A	1.75 x ULN
Creatinine	mg/dL	N/A	1.0 x ULN
Creatine kinase	U/L	N/A	< 2.5 x ULN
eGFR	mL/min/1.73m2	60	N/A
Total Protein	g/L	≥ 0.85 x LLN	≤ 1.25 x ULN
Albumin	g/L	≥ 0.85 x LLN	≤ 1.25 x ULN
Total Bilirubin	mg/dL	N/A	1.25 x ULN
Direct Bilirubin	mg/dL	N/A	1.25x ULN
ALP	U/L	N/A	1.5 x ULN
AST	U/L	N/A	1 x ULN
ALT	U/L	N/A	1 x ULN
GGT	U/L	N/A	1.5 x ULN
Lactate Dehydrogenase	U/L	0.9x LLN	1.1 x ULN
Prothrombin time INR	INR	1.0 x LLN	1.0 x ULN
Cholesterol	mg/dL	N/A	1.2 x ULN

Test	Unit	Acceptable Inclusion Range	
		Low	High
HDL Cholesterol	mg/dL	0.9x LLN	N/A
LDL Cholesterol	mg/dL	N/A	1.25 x ULN
Haemoglobin	g/dL	0.9x LLN	1.1.x ULN
Platelets	10E9/L	0.9x LLN	1.1 x ULN
White Blood Cells	10E9/L	0.9x LLN	1.1 x ULN
Neutrophils	10E9/L	1.0 x LLN	1.0x ULN
Lymphocytes	10E9/L	1.0 x LLN	1.0 x ULN
Monocytes	10E9/L	N/A	1.2 x ULN
Eosinophils	10E9/L	N/A	1.0 x ULN
Basophils	10E9/L	N/A	2.0 x ULN
C-reactive protein (CRP)	mg/L	N/A	1.0 x ULN
Troponin-T (high sensitivity)	ng/L	N/A	< 12
Protein (dipstick)		N/A	1+ or 30mg/dL
Ketones (dipstick)		N/A	<3+ or <80mg/dL
Red Blood Cells (MCS)		N/A	<20*
White Blood Cells (MCS)		N/A	<10
Casts (MCS)		N/A	<2/high power field

*A result ≥ 20 is acceptable for female volunteers currently menstruating.

APPENDIX 2: MALARIA CLINICAL SCORE

Symptoms	Clinical Score/CTCAE grade			
	Absent (0)	Mild (1)	Moderate (2)	Severe (3)
		CTCAE 1	CTCAE 2	CTCAE 3
Headache		Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL
Myalgia		Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL
Arthralgia		Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL
Fatigue		Fatigue relieved by rest	Fatigue not relieved by rest; limiting instrumental ADL	Fatigue not relieved by rest; limiting self-care ADL
Malaise		Uneasiness or lack of well-being	Uneasiness or lack of well-being; limiting instrumental ADL	Uneasiness or lack of well-being limiting self-care ADL
Chills		Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics
Sweating/hot spells		Mild sweating/hot spells not affecting ADL	Moderate sweating/hot spells; narcotics indicated	Severe or prolonged, not responsive to narcotics
Reduced appetite		Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g. inadequate oral caloric and/or fluid intake); tube feeding or TPN indicated
Nausea		Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalisation indicated
Vomiting		Intervention not indicated	Outpatient IV hydration; medical intervention indicated	Tube feeding, TPN, or hospitalisation indicated
Abdominal discomfort		Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL
Fever		38.0-38.9°C	≥39.0-39.9°C	≥40.0°C
Tachycardia		HR ≥100 Asymptomatic, intervention not indicated	HR ≥100 Symptomatic; non-urgent medical intervention indicated	HR ≥100 Urgent medical intervention indicated
Hypotension		SBP ≤80 Asymptomatic, intervention not indicated	SBP ≤80 Symptomatic; non-urgent medical intervention indicated	SBP ≤80 Urgent medical intervention indicated
Total Score				
Maximum 3 x 14 = 42				
Indications for malaria treatment:				

Symptoms	Clinical Score/CTCAE grade
• Volunteer has a clinical score ≥6, or • Volunteer has ≥1 CTCAE grade 3 (severe) AE deemed possibly related to malaria and not self-resolved or relived with concomitant medications, or • Volunteer experiences ≥1 SAE, or • The Principal Investigator or delegate considers it necessary for volunteer safety. In this situation, the Principal Investigator or delegate will notify the medical monitor prior to initiating treatment.	