Life Sciences, a division of SGS Belgium NV

Medicines for Malaria Venture

REVISED CLINICAL STUDY PROTOCOL

A single centre, randomised, double-blind, placebo-controlled, Phase Ib study to evaluate the safety, tolerability and chemoprotective antimalarial activity of P218 against controlled human malaria infection with *Plasmodium falciparum* sporozoite challenge in non-immune healthy adult volunteers

Product	P218
Protocol Number	MMV_P218_17_01
EudraCT Number	2018-003004-39
Clinical Phase	Ib
Clinical Indication	Malaria infection
Issue Date (Version)	20-mar-2019 (Final 2.0) (Final Approved Protocol dated 25-sep-2018 [Final 1.0])

Sponsor	Medicines for Malaria Venture (MMV)
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This study will be conducted in compliance with this protocol, the ICH Note for Guidance on Good Clinical Practice (CMPM/ICH/135/95) and with the applicable regulatory requirement(s).

SIGNATURES

Signature of Sponsor Representative

Title: **A single centre, randomised, double-blind, placebo-controlled, Phase Ib study to evaluate the safety, tolerability and chemoprotective antimalarial activity of P218 against controlled human malaria infection with *Plasmodium falciparum* sporozoite challenge in non-immune healthy adult volunteers**

'This Revised Clinical Study Protocol has been reviewed and approved by the Sponsor in order to ensure compliance with Good Clinical Practice.'

Name:	Farouk Chughlay, MD
Signature:	p.o. Stephan Chalon MD, PhD
Date:	March 21, 2019

Name:	Myriam El Gaaloul, PharmD
Signature:	
Date:	21-March-2019.

Signature of Investigator

Title: **A single centre, randomised, double-blind, placebo-controlled, Phase Ib study to evaluate the safety, tolerability and chemoprotective antimalarial activity of P218 against controlled human malaria infection with *Plasmodium falciparum* sporozoite challenge in non-immune healthy adult volunteers**

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'I have read this Revised Clinical Study Protocol and agree that it contains all information necessary for proper conduct of the study. I will carry out the study as outlined herein and will complete the study within the designated time.'

Signature:

Date: 22MAR2019

PROTOCOL HISTORY

Protocol History[a]

Medicines for Malaria Venture (MMV) – MMV_P218_17_01

Document	Issue Date	Amendment Type	Comments
Final Approved Protocol [Final 1.0]	25-sep-2018	Not applicable	-
Amendment 1	20-mar-2019	Substantial	Revised Clinical Study Protocol 2.0 Based on new *in vitro* experiments suggesting that P218 inhibits all stages of intra-hepatocytic development and the encouraging preliminary clinical data obtained so far for Cohort 2, the Sponsor believes that proof of pharmacology has been achieved. Therefore, the Sponsor would like to decrease the dose of P218 in Cohort 3 from 1000 mg to 100 mg, to be administered at D1 and D3 (instead of D3 and D5), to better estimate the minimal inhibitory exposure of P218 required for liver activity.

[a] This overview only lists general amendments to the protocol. Site- and country-specific amendments to the protocol are not included.

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PROTOCOL AMENDMENT 1: SUMMARY OF CHANGES

Overall Reason for Amending the Protocol: This amendment was created to make adaptations to Cohort 3.

To date, two cohorts have been successfully completed.

The primary objective of Cohort 1 is to assess safety and tolerability of two single doses of 1000 mg administered 48 hours apart in healthy volunteers (D1 and D3). 1000 mg P218 is the highest dose tested to date in clinical trials. At the completion of Cohort 1, the SRT reviewed the safety/tolerability and PK data of Cohort 1 and unanimously agreed that there were no concerns or unexpected findings regarding the safety or PK data for Cohort 1 and the study should proceed to Cohort 2.

The primary objective of Cohort 2 is to assess the chemoprotective activity of P218 in non-immune healthy volunteers after the *P. falciparum* sporozoite challenge agent has been administered through direct venous inoculation. Two single doses of 1000 mg P218 were administered 48 hours apart (on D1 and D3), as for Cohort 1, in order to interfere with the liver stage of the parasite. The cohort included 3 placebo and 9 active treatments. The cohort is still blinded, yet 3 subjects had breakthrough of parasitaemia at between Days 8 and 12, and 9 subjects did not have results which met the definition of positive PCR parasitemia up to Day 28. Hence, it seems there is no breakthrough in subjects treated with P218 if it is assumed that the three subjects with positive parasitaemia received the placebo, leading to the conclusion that P218 has chemoprotective activity.

Based on new *in vitro* experiments suggesting that P218 inhibits all stages of intra-hepatocytic development and the encouraging preliminary clinical data obtained so far for Cohort 2, the Sponsor believes that proof of pharmacology has been achieved. Therefore, the Sponsor would like to decrease the dose of P218 in Cohort 3 from 1000 mg to 100 mg, to be administered at D1 and D3 (instead of D3 and D5), to better estimate the minimal inhibitory exposure of P218 required for liver activity.

Changes are summarised below together with a rationale for each change.

1. Change of Study Design

Rationale:

In order to better estimate the minimal inhibitory exposure, the dose in Cohort 3 was de-escalated from 1000 mg to 100 mg and days of administration were changed from D3 and D5 to D1 and D3. The cohort will consist of two sequential subgroups, each with 6 subjects. In each subgroup, at least 1 subject will receive placebo and the remaining subjects will receive two 100 mg doses of P218 on D1 and D3, i.e. at the same times as Cohort 2. A secondary objective has been added to reflect the above mentioned objective.

Applicable Section(s)	Description of Changes
Synopsis	A secondary objective and a secondary endpoint were added. The overview of study design was described for Cohorts 2 and 3 together, and adapted for Cohort 3 according to the rationale. The text '50 mg capsules of P218' was added to the description of the IMP (P218/Placebo), Dose, Mode of Administration. The study/Treatment Duration was adapted. A section was added for the PK/PD analysis. The PK/PD population was added. Determination of Sample Size was amended.
Time and Events Schedule	The Time and Events Schedule for Cohort 3 was deleted. The Time and Events Schedule for Cohort 2 was adapted to describe Cohorts 2 and 3 together.
Section 2.1.2	A secondary objective was added.
Section 2.2	A secondary endpoint was added.
Section 3.1	The overview of study design is described for Cohorts 2 and 3 together, and adapted for Cohort 3 according to the rationale.
Section 3.2	The rationale for dose selection was updated.
Section 5	The text '2x50 mg capsules of P218' was added and timing of snacks, lunch and dinner after the study drug administration was amended.
Section 7.1	Timing of Cohort 3 was aligned with the timing of Cohort 2.
Section 7.3.3	This section was added for the PK/PD analysis.
Section 9.1.2	The PK/PD population was added (and 'PD' to the abbreviation list).
Section 9.2	Determination of Sample Size was amended.

2. Practical Aspects

Rationale:

Practical Aspects

Applicable Section(s)	Description of Changes
Time and Events Schedule	A footnote was added for all cohorts that the assessment for serum folate on D-1 can be done at either D-3 or D-1, upon Investigator's decision.

3. Administrative Change

Rationale:

Administrative Change

Applicable Section(s)	Description of Changes
Study Administrative Structure and Investigators	Charles Stoyanov was replaced by Nicola Kerr as Clinical Operations Lead.

4. Clarifications

Rationale:

Clarifications

Applicable Section(s)	Description of Changes
Synopsis Section 5.2	The description of the antimalarial rescue medication was clarified: treatment success, defined as one negative qPCR outcome for asexual parasites, means that 0 parasites per mL were detected
Section 7.3.2	In the description of the malaria clinical score, it was specified that if the PCR results for *P. falciparum* are between 0 and < 250 asexual parasites per mL (i.e. 'negative' was deleted here) at the time of the onset of the event, the usual AE/SAE reporting procedures and criteria will apply, and the event will not be classified as an inoculum-related event. For completeness, a positive *P. falciparum* PCR was defined as ≥ 250 asexual parasites per mL.
Time and Events Schedule	A footnote was deleted for Cohort 1 at 48 hours and for Cohorts 2 and 3 at 50 hours, as this footnote only applies to safety lab assessments prior to first IMP administration.

5. Typographical Corrections

Rationale:

Typographical Corrections

Applicable Section(s)	**Description of Changes**
Synopsis	Exclusion criterion 22 was aligned with body of the protocol.
	'ski' was corrected to 'skin' in the description of physical examinations.
Table of Contents	The footer was corrected.
Section 7.4.5	'ski' was corrected to 'skin'
Attachment 1	A more applicable example of clinical score card for malaria was inserted. The total score and corresponding text was deleted as this will not be used as a threshold for commencement with IMP in this study.

PROTOCOL SYNOPSIS

Study Title	A single centre, randomised, double-blind, placebo-controlled, Phase Ib study to evaluate the safety, tolerability and chemoprotective antimalarial activity of P218 against controlled human malaria infection with *Plasmodium falciparum* sporozoite challenge in non-immune healthy adult volunteers		
Product	P218	**Clinical Phase**	Ib
Protocol Number	MMV_P218_17_01	**Indication**	Malaria infection
EudraCT Number	2018-003004-39		

Sponsor	Medicines for Malaria Venture (MMV)
Sponsor Medical Director	Farouk Chughlay, MD
Sponsor Clinical Lead	Myriam El Gaaloul, PharmD
Clinical Centre	SGS Life Sciences, Clinical Pharmacology Unit, Lange Beeldekensstraat 267, 2060 Antwerpen, Belgium

Objectives:

For Cohort 1, the primary objective is to assess the safety and tolerability of two single doses of 1000 mg P218 administered 48 hours apart in healthy adult volunteers.

For Cohorts 2 and 3, the primary objective is to assess the chemoprotective activity of P218 in *Plasmodium falciparum* (*P. falciparum*) controlled human malaria infection (CHMI) in non-immune healthy volunteers after *P. falciparum* sporozoites (PfSPZ) Challenge through direct venous inoculation (DVI).

Secondary objectives are:

- To assess the pharmacokinetic (PK) profile of P218, and in Cohort 1 only also of its main metabolites, up to 8 days after first P218 administration to healthy adult volunteers;
- To establish the pharmacokinetic/pharmacodynamic (PK/PD) relationship between P218 plasma concentration and its chemoprotective activity using blood stage parasitaemia as a surrogate in non-immune healthy volunteers in a CHMI PfSPZ Challenge model;
- To assess the safety and tolerability of P218 in non-immune healthy volunteers in a CHMI PfSPZ Challenge model (Cohorts 2 and 3);
- To assess the safety and tolerability of PfSPZ Challenge in non-immune healthy volunteers before and after P218 administration during CHMI (Cohorts 2 and 3).

There is no formal hypothesis testing as this is an exploratory study.

Endpoints:

For Cohort 1, the primary endpoint is the incidence, severity and relationship to P218 of observed or self-reported treatment-emergent adverse events (TEAEs) during the 9-day observation period without PfSPZ Challenge.

For Cohorts 2 and 3, the primary endpoint is the cohort-specific, geometric mean time to parasitaemia based in each case on the time elapsed between PfSPZ Challenge DVI and first quantitative polymerase chain reaction (qPCR) outcome equal or greater than 250 asexual parasites per mL, with a maximum of 28 days in the absence of parasitaemia.

Secondary endpoints are:

- Estimation of the following PK parameters for P218, and in Cohort 1 only, also for its major metabolites (P218-OH, P218-beta-acyl-glucuronide, P218-beta-acyl-glucuronide-OH), over

8 days after first investigational medicinal product (IMP) administration using non compartmental methods:
  - Area under the plasma concentration-time curve during the dosing interval (AUC_{τ}) = AUC during the first 48 hours after dosing ($AUC_{0\text{-}48h}$) after each administration (i.e. $AUC_{0\text{-}48h}$ and $AUC_{48\text{-}96h}$);
  - AUC from 48 hours after dosing to infinity ($AUC_{48h\text{-}inf}$), calculated from $AUC_{48h\text{-}t} + (C_t/\lambda_z)$, where C_t is the last observed quantifiable concentration and λ_z the first order terminal rate constant;
  - AUC calculated between 48 hours after dosing and last quantifiable concentration ($AUC_{48h\text{-}last}$);
  - Total plasma clearance (CL/F) and apparent volume of distribution (Vz/F) after the second administration (not for metabolites);
  - Observed maximum plasma concentration (C_{max}) and the time to reach the maximum concentration after drug administration (t_{max}) after each administration;
  - The terminal elimination half-life ($t_{1/2}$) after the second administration;
  - The accumulation ratio (R_{ac}), calculated as $AUC_{48\text{-}96h}/AUC_{0\text{-}48h}$;
  - Metabolic ratio of AUC_{τ} and $AUC_{48h\text{-}inf}$ for P218 β-acyl glucuronide, P218 OH and P218-OH β-acyl glucuronide over parent P218 (Cohort 1 only).
- The minimal inhibitory concentration of P218 needed for chemoprotective activity will be predicted from the P218 PK/PD model;
- The incidence, severity and relationship to P218 of observed or self-reported, TEAEs during the 35-day observation period with PfSPZ Challenge (Cohorts 2 and 3 only);
- The incidence and severity of observed (i.e. Malaria signs and symptoms graded by the Malaria clinical score) or self-reported inoculum-related events and adverse events (AEs) considered PfSPZ Challenge-related (Cohorts 2 and 3 only);
- The Malaria clinical score at the time of introduction of rescue therapy (Cohorts 2 and 3 only);
- Changes from baseline in folate levels, haematology, clinical chemistry and urinalysis parameters, vital signs and electrocardiogram (ECG) parameters.

Overview of Study Design:

This is a single centre, randomised, double-blind, placebo-controlled Phase Ib study. Thirty-two healthy men and women aged 18 to 45 years will be enrolled in 3 cohorts of 8, 12 and 12 subjects. A subject may be enrolled in one cohort only and will be randomised in a 3:1 ratio, to receive two consecutive administrations of either P218 or placebo. Enrolment in cohorts will proceed sequentially, to facilitate review of Cohort 1 and Cohort 2 data by a Safety Review Team (SRT) before populating Cohort 2 and Cohort 3, respectively.

Cohort 1

Cohort 1 will consist of 2 subgroups of subjects, to be enrolled sequentially: subgroup 1 will be composed of 2 subjects: one to receive two 1000 mg doses of P218 48 hours apart and one to receive placebo; subgroup 2 will be composed of 6 subjects: five to receive two 1000 mg doses of P218 48 hours apart and one to receive placebo. Subjects in subgroup 2 will not be treated until 24 hours after second IMP administration in the last subject of subgroup 1 and only upon decision of the PI after review of available safety data of subgroup 1.

Subjects will be admitted to the clinical unit in the morning of Day -1 and will be confined to the unit for 3 days thereafter (until Day 4, i.e. 36 hours after second IMP administration), for close safety monitoring and PK assessments. First administration of 1000 mg of P218 or placebo will take place on Day 1. Second administration of 1000 mg of P218 or placebo will take place 48 hours later, on Day 3. After discharge from the clinical unit on Day 4, subjects will be followed up with daily ambulatory visits to the clinical unit up to Day 9.

Progression to Cohort 2 will be assessed by an SRT, who will review safety and tolerability after data up to Day 9 for Cohort 1 are available.

Cohorts 2 and 3

Cohort 2 will consist of three subgroups of subjects, to be enrolled sequentially: subgroup 1 will be composed of 2 subjects: one to receive two 1000 mg doses of P218 48 hours apart and one to receive placebo; subgroups 2 and 3 will be composed of 5 subjects each: four to receive two 1000 mg doses of P218 48 hours apart and one to receive placebo. Subjects in subgroup 2 will not be treated until 24 hours

after second IMP administration in the last subject of subgroup 1. In subgroup 2 and subgroup 3, subjects will only be inoculated upon decision of the PI after review of available safety data of the previous subgroup, i.e. subgroup 1 and subgroup 2, respectively.

Cohort 3 will consist of two subgroups, each with 6 subjects, to be enrolled sequentially. Treatment will be allocated in a ratio of 3 active to 1 placebo. In each subgroup, at least 1 subject will receive placebo and the remaining subjects will receive two 100 mg doses of P218 48 hours apart. Subjects in subgroup 2 will not be inoculated until 24 hours after second IMP administration in the last subject of subgroup 1.

Subjects will be admitted to the clinical unit in the morning of Day -1 and will be confined to the unit until 12 days post the PfSPZ Challenge on Day 1 (i.e. until Day 13), for close safety monitoring and PK assessments. Each subject will be administered 3,200 *P. falciparum* sporozoites by DVI. First administration of P218 or placebo will take place 2 hours after PfSPZ Challenge inoculation. Second administration of P218 or placebo will take place 48 hours after first administration of P218or placebo, on Day 3.

Safety, parasitaemia and Malaria signs and symptoms (Malaria clinical score) will be assessed as of Day 7, i.e. during confinement in the unit until Day 13 and on daily ambulatory visits to the clinical unit as of Day 14, until development of positive parasitaemia or until Day 28 otherwise. If positive parasitaemia is confirmed, the subject will receive rescue therapy and will continue to be monitored daily at the clinical unit until treatment success. Upon treatment success, daily visits to the clinical unit are stopped, except for subjects that first need to complete the 3-day rescue treatment regimen and associated assessments. All subjects will be assessed again for parasitaemia at the end-of-study (EOS) visit on Day 35. Subjects not developing positive parasitaemia until Day 28 will receive rescue therapy on that day. Instead of daily monitoring at the clinical unit until treatment success, these subjects will only be assessed again for parasitaemia at the EOS visit on Day 35. All subjects that receive rescue therapy will be asked non leading questions to determine the occurrence of any AEs approximately two weeks after initiation of the rescue treatment, either during a planned study visit or by phone in case there is no planned study visit at that time.

Antimalarial rescue therapy may be initiated whenever deemed necessary by the Investigators, e.g. if there is a concern regarding the safety of a study subject or if a study subject decides to withdraw from the study. Therapy may be amended according to the treating physician if the patient does not respond to treatment or the condition worsens.

Progression to Cohort 3 will be assessed by the SRT, who will review safety, parasitaemia and Malaria signs and symptoms after data up to Day 35 for Cohort 2 are available, or after successful completion of the last rescue treatment, whichever is later.

Study Population:

Approximately 32 subjects are planned to be enrolled in one of 3 cohorts of 8, 12 and 12 subjects. A subject may be enrolled in one cohort only. Subjects will be randomised within each cohort in a 3:1 ratio to receive two consecutive administrations of either P218 or placebo.

Eligibility Criteria:

Inclusion Criteria:

1. Informed Consent Form signed voluntarily before any study-related procedure is performed, indicating that the subject understands the purpose of and procedures required for the study and is willing to participate in the study, including administration of rescue treatment.
2. Male or female, between 18 and 45 years old (extremes included) at screening.
3. Body weight of at least 50 kg and a body mass index (BMI) of 19 to 30 kg/m^2 (extremes included).
4. Good general health without clinically relevant medical illness, physical exam findings including vital signs, and laboratory abnormalities as determined by the investigator.
5. Willing to adhere to the prohibitions and restrictions specified in this protocol, including willingness to stay confined to the inpatient unit for required duration and willingness to avoid to travel outside of Benelux during the study period.
6. Female subjects should fulfil one of the following criteria:
   a. At least 1 year post-menopausal (amenorrhea >12 months and follicle stimulating hormone (FSH) >30 mIU/mL) prior to screening;
   b. Surgically sterile (bilateral oophorectomy, hysterectomy or tubal ligation);

c. Will use contraceptives as outlined in inclusion criteria 7 and 8.

7. Female subjects of childbearing potential must agree to the use of a highly effective method of birth control from screening visit to until 40 days after the last dose of IMP (covering a full menstrual cycle of 30 days starting after 5 half-lives of last dose of IMP).
   Note: Highly effective birth control methods include: combined (estrogen and progestogen containing) oral/intravaginal/transdermal 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.
8. Male subjects who are sexually active with a female partner of childbearing potential must agree to the use of an effective method of birth control from the day of the first IMP dose until 100 days thereafter (covering a full sperm cycle of 90 days starting after 5 half-lives of last dose of IMP).
   Note: Medically acceptable methods of contraception that may be used by the subject and/or partner include sterilization and vasectomy or a double barrier option combining oral contraceptive, contraceptive vaginal ring, contraceptive injection, intrauterine device or etonogestrel implant.
9. Female subject has a negative pregnancy test at screening and upon admission in the clinical unit.
   Note: Pregnancy testing will consist of a serum β-human chorionic gonadotropin (β-HCG) test at screening and urine β-HCG tests at other visits, in all women.

Inclusion Criteria - CHMI specific:

10. Different ways of being reachable 24/7 (e.g. by mobile phone, regular phone or electronic mail) during the whole study period.

Exclusion Criteria:

1. Nursing (lactating) women.
2. Participation in any other clinical drug or vaccine study within 30 days (or five half-lives for drugs) preceding the first dose of IMP (whichever is longer), or plans to participate in other investigational drug or vaccine research during the study period.
3. Blood product donation to any blood bank during the 8 weeks (whole blood) or 4 weeks (plasma and platelets) prior to admission in the clinical unit.
4. ECG outside normal range and deemed clinically relevant by the investigator. Examples of clinically significant ECG abnormalities for this study include:
   a. PR-interval >220 ms;
   b. QRS-complex >120 ms;
   c. QT interval corrected according to Bazett's formula (QTcB) or QT interval corrected according to Fridericia's formula (QTcF) >450 ms;
   d. Pathologic Q wave;
   e. Significant ST-T wave changes;
   f. Left or right ventricular hypertrophy;
   g. Non-sinus rhythm except isolated premature atrial contractions and ventricular extrasystole <2 per 10 s ECG lead;
   h. Incomplete left bundle branch block, or complete or intermittent right or left bundle branch block;
   i. Second or third degree A-V heart block.
5. Seropositive human immunodeficiency virus (HIV) (antibody and antigen), hepatitis B virus (HBV) (hepatitis B surface antigen [HBsAg]) or hepatitis C virus (HCV) (antibody) tests.
6. History or presence of diagnosed food or known drug allergies (including but not limited to allergy to any of the antimalarial rescue medications to be used in the study), or history of anaphylaxis or other severe allergic reactions.
   Note: Subjects with seasonal allergies/hay fever, house dust mite or allergy to animals that are untreated and asymptomatic at the time of dosing can be enrolled in the study.
7. History of convulsion or severe head trauma.
   Note: A medical history of a single febrile convulsion during childhood is not an exclusion criteria.
8. 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, subjects 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. Subjects 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.

9. A medical, occupational or family problem as a result of alcohol or illicit drug abuse during the past 12 months or current alcohol or illicit drug abuse or addiction (positive alcohol breath test or positive drug screen for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine or opiates at screening or upon check-in at the clinical unit).
Note: Excessive use of alcohol is an intake of >21 units per week for males and >14 units per week for females where one alcohol unit is defined as 10 mL or 8 g of pure alcohol. A single unit is equal to one 25-mL (single) measure of whisky (alcohol by volume [ABV] 40%), or a third of a pint of beer (190 mL; ABV 5-6%) or half a standard (175 mL) glass of wine (ABV 12%).
10. Subjects are non-smokers or ex-smokers for more than 90 days prior to screening or smoke no more than 5 cigarettes per day. If users of nicotine products (i.e. spray, patch, e-cigarette, etc.) they should use the equivalent of no more than 5 cigarettes per day. Subjects must agree to abstain from smoking while in the unit.
11. Use of any prescription drugs, herbal supplements (e.g. St John's Wort) or over the counter medication within 7 days or five half-lives (whichever is longer) prior to the first IMP administration, or an anticipated requirement for the use of these during the course of the study.
Note: If necessary, the incidental use of non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol (2g/day, 10 gr/week), vitamins and topical treatments may be acceptable after approval by the study Sponsor and will be documented in the eSource system. The use of nutritional supplements during this time that are not believed to have the potential to affect subject safety nor 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.
12. Any surgical or medical condition possibly affecting drug absorption (e.g. cholecystectomy, gastrectomy, bowel disease), distribution, metabolism or excretion.
13. Any history of gallbladder disease, including cholecystitis and/or cholelithiasis.
14. History of megaloblastic anaemia or folate deficiency.
15. Personnel (e.g. investigator, sub-investigator, research assistant, pharmacist, study coordinator or anyone mentioned in the delegation log) directly involved in the conduct of the study.
16. Any condition that in the opinion of the investigator would jeopardize the safety or rights of a person participating in the trial or would render the person unable to comply with the protocol.

Exclusion Criteria - CHMI specific:

17. Glucose-6-phosphate dehydrogenase (G6PD) deficiency (due to possible hemolysis induced by primaquine treatment at study end in G6PD deficient subjects).
18. Personal history of malaria.
19. Volunteer has travelled to or lived in a malaria-endemic area for more than 4 weeks during the 12 months prior to first IMP administration, or spent any time in an endemic area during the 4 weeks prior to first IMP administration.
20. Plans to travel to malaria-endemic region during the study period up to last follow-up visit.
21. Previous participation in any malaria vaccine or CHMI study.
22. Falling in moderate or higher risk category for a fatal or non-fatal cardiovascular event within 10 years (≥5%) determined by a validated risk estimation system e.g. SCORE.
23. Use of medications known to interact with atovaquone-proguanil (Malarone®), artemether-lumefatrine (Riamet®) or primaquine (Primaquine®) such as cimetidine, metoclopramide or antacids, or an anticipated requirement for the use of these at any point during the study period.
24. Use of systemic antibiotics with known antimalarial activity within 30 days (or 5 half-lives whichever is longer) of first IMP administration (e.g. trimethoprim sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones or azithromycin) or an anticipated requirement for the use of these during the study period.
25. Receipt of blood or blood-derived products (including immunoglobulin) within 3 months prior to screening. Receipt of packed red blood cells given for an emergent indication in an otherwise healthy person, and not required as ongoing treatment is not exclusionary (for example packed red blood cells emergently given during an elective surgery).

Note: In case of an out-of-range clinical laboratory test, vital sign or ECG value that will determine a subject's eligibility, or in case of a positive drug screen, a retest or expert evaluation can be requested. Results of any retest must be available prior to inoculation. The result of the retest will be considered for subject eligibility at the investigator's discretion. Subjects can be rescreened at the discretion of the investigator.

IMP (P218/Placebo), Dose, Mode of Administration:

P218 capsules for oral administration will be supplied by the Sponsor. Each capsule will contain 50 mg or 250 mg P218.

To preserve blinding, placebo capsules will be matched to the drug product with regard to appearance and taste. They are contained in the same packaging as the drug product.

On each scheduled dosing, subjects will take simultaneously 4 capsules (either placebo or a total of 1000 mg P218) p.o in Cohorts 1 and 2 or 2 capsules (either placebo or a total of 100 mg P218) p.o. in Cohort 3. Subjects will be asked to take the study drugs under the supervision of the clinical staff. Study drug administration will be done with 240 mL of non-carbonated water and in a fasted state (overnight fast for at least 8 hours; intake of water will be allowed until 1 hour before the administration of the study drugs). After intake of the study drugs, the subjects will be asked to maintain an upright position for at least 15 minutes. Thereafter, they will be asked to remain seated in bed for 2 hours. Fasting conditions will be maintained for 2 hours, including drinking water that will be allowed from 2 hour post dose onwards (ad libitum but approximately 1500mL/24 hours).

PfSPZ Challenge, Dose, Mode of Administration:

The PfSPZ Challenge consists of aseptic, cryopreserved *P. falciparum sporozoites* used for CHMI trials and is produced and provided by the biotechnology company, Sanaria (USA). Each cryovial contains 15,000 or 50,000 sporozoites.

The PfSPZ Challenge (3,200 *P. falciparum* sporozoites per subject) will be administered intravenously by DVI. The study staff administering the PfSPZ Challenge will wear gloves and eye protection. Advanced life support drugs and resuscitation equipment will be immediately available in the event of any subjects experiencing an anaphylactic reaction to the challenge.

Antimalarial Rescue Medication:

The clinical unit will be responsible for acquiring the anti-malarial drugs, Riamet®, Malarone® and Primaquine®:

- Subject will be prescribed with Riamet® (20 mg artemeter and 120 mg lumefantrine) to ensure parasite clearance prior to the end-of-study evaluation. Tablets for oral use will be taken over 3 days. The first dose should be taken as soon as possible and should be followed by five further doses approximately 8, 24, 36, 48 and 60 hours after the first dose. Drug administration should be immediately followed by a meal or drinks rich in fat (e.g. milk).
- If an intolerance or contraindication to Riamet® develops, Malarone® (250 mg atovaquone and 100 mg proguanil hydrochloride; tablets for oral use) will be administered for 3 consecutive days.
- To ensure complete clearance of gametocytes (a low probability event in this study due to early treatment at low parasitemia levels), subjects will receive a single dose of Primaquine® (26.4 mg primaquine phosphate equivalent to 15 mg primaquine base; tablets for oral use, registered product in Canada) on the first day of rescue treatment or on Day 28 together with rescue treatment, for subjects who do not develop positive parasitaemia until this day.

The PfSPZ Challenge product consists of a strain *P. falciparum* sporozoites used for CHMI trials that is known to be sensitive to the rescue treatment described above.

Rescue treatment will be administered and monitored on the occasion of daily ambulatory visits to the clinical unit. The required anti-malarial drug intakes 36 and 60 hours after the first dose, can be done at home. Subjects will be notified by a study nurse via phone/text message/e-mail when it is time for dosing and must confirm study drug intake. Thick blood smear (TBS) microscopy and qPCR assessments of parasitaemia will be carried out daily until treatment success (defined as one negative qPCR outcome for asexual parasites, i.e. 0 parasites per mL, confirmed by TBS microscopy). Upon treatment success, daily visits to the clinical unit are stopped, except for subjects that first need to complete the 3-day rescue treatment regimen and associated assessments. All subjects will be assessed for parasitaemia at the EOS visit on Day 35.

In subjects who do not develop positive parasitaemia until Day 28 of the study, rescue treatment will be initiated on Day 28. Instead of daily qPCR, TBS and Troponin I assessments until treatment success, for these subjects only a final qPCR will be performed at the EOS visit on Day 35.

All subjects that receive rescue therapy will be asked non leading questions to determine the occurrence of any AEs approximately two weeks after initiation of the rescue treatment, either during a planned study visit or by phone in case there is no planned study visit at that time.

All subjects must consent to rescue treatment. Even in case of withdrawal from the study, all challenged subjects are to receive rescue treatment as soon as possible, including all appropriate visits and assessments, and phone call as required.

Study/Treatment Duration:

In Cohort 1, each subject will be in the study for approximately 1 month and 1 week (37 days) maximum, including a screening period of up to 28 days and a follow-up period until 9 days after first IMP administration on Day 1. IMP will be administered twice; on Days 1 and 3.

In Cohorts 2 and 3, each subject will be in the study for approximately 2.5 months (73 days) maximum, including a screening period of up to 28 days, a follow-up period until 35 days after PfSPZ DVI and a possible follow-up call approximately 2 weeks after start of rescue treatment, i.e. at 45 days after PfSPZ DVI at the latest. IMP will be administered twice; on Days 1 and 3.

Assessments:

Pharmacokinetics

Plasma samples for determination of P218 concentration, and concentration of its major metabolites P218 β-acyl-glucuronide, P218-OH β-acyl-glucuronide and possibly P218 OH in Cohort 1 only, will be analysed by Swiss BioQuant laboratory on behalf of MMV using a validated high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method at scheduled time points.

Pharmacokinetic calculations will be performed by SGS-Life Sciences using Phoenix WinNonLin 8.0 or higher (Pharsight Corporation, Palo Alto, CA, USA).

The following parameters, where appropriate, will be determined for P218, and in Cohort 1 only also for its major metabolites (P218-OH, P218-beta-acyl-glucuronide, P218-beta-acyl-glucuronide-OH) using individual concentration data in plasma, according to the definitions and methods of calculation below:

- AUC_{τ} = $AUC_{0\text{-}48h}$ after each administration (i.e. $AUC_{0\text{-}48h}$ and $AUC_{48\text{-}96h}$);
- $AUC_{48h\text{-}inf}$, calculated from $AUC_{48h\text{-}t} + (C_t/\lambda_z)$;
- $AUC_{48h\text{-}last}$;
- CL/F and Vz/F after the second administration (not for metabolites);
- C_{max} and t_{max} after each administration;
- $t_{1/2}$ after the second administration;
- R_{ac}, calculated as $AUC_{48\text{-}96h}/AUC_{0\text{-}48h}$;
- Metabolic ratio of AUC_{τ} and $AUC_{48h\text{-}inf}$ for P218 β-acyl glucuronide, P218 OH and P218-OH β-acyl glucuronide over parent P218 (Cohort 1 only).

Additional pharmacokinetic parameters may be calculated as appropriate.

Efficacy

Parasitaemia

The assessment of malaria parasitology by TBS and qPCR will be as follows:

- Parasite density, expressed as the number of parasites per microliter of blood will be measured using the routine diagnostic TBS microscopy method for parasite count employed by the centre of excellence for tropical medicine (ITM) as per relevant standard operating procedure and Laboratory Manual. The reading will be considered positive if 2 unambiguous malaria structures are seen in at least 0.5µl of blood and the observation is confirmed by a second expert malaria microscopist.
- *var*ATS (the acidic terminal segment in *Plasmodium var* genes) targeted qPCR assay of parasite load will be performed in accordance with the ITM standard operating procedure and the Laboratory Manual. Method validation and external quality assessment (EQA) of the outcomes will be carried out at the Department of Laboratory Medicine, School of Medicine, University of Washington (Dr. Sean Murphy) using 18S rRNA targeted qRT-PCR methodology described in a dedicated Validation Plan. Given the higher sensitivity of qPCR compared to microscopy, this method will be used to confirm aparasitaemia after definitive antimalarial therapy for all subjects. A subject will be considered cured following completion of the course of rescue antimalarial therapy and after qPCR results are obtained with values below the limit of detection.

The results of the TBS microscopy and qPCR at the ITM will be available in approximately 24 hours.

Malaria Clinical Score

An inoculum-related event is a sign or symptom associated with malaria infection (confirmed by a positive *P. falciparum* PCR [defined for the purpose of this study as ≥ 250 asexual parasites per mL] at the onset of the event) that is of expected intensity, frequency and duration for the individual subject in the context of this study.

Prevention of expected signs and symptoms associated with malaria infection form part of the efficacy evaluation of the study. If the presence of *P. falciparum* malaria is confirmed by positive PCR at the time of onset of these signs/symptoms and if, in the Investigator's opinion, the signs/symptoms are of the expected intensity, frequency and duration for the individual subject in the context of this study, then the events will be classified as inoculum-related events and reported as such in the final clinical study report.

Inoculum-related events meeting the classification criteria for SAEs or AEs of special interest should be subjected to standard expedited reporting procedures of these events.

The following malaria signs/symptoms will not be classified as inoculum-related events:

- If the PCR results for *P. falciparum* are between 0 and < 250 asexual parasites per mL at the time of the onset of the event, usual AE/SAE reporting procedures and criteria will apply, and the event will not be classified as an inoculum-related event.
- Observed malaria symptoms or signs that are of greater intensity, frequency or duration than would be expected in the context of this study, will be regarded as medically important events and must be reported promptly (i.e. in an expedited manner) to the Sponsor using an SAE report form, and will not be classified as inoculum-related events.

Final classification of signs and symptoms as inoculum-related event or AE will occur after PCR results are available.

Safety

Adverse Events

Adverse events will be monitored continuously from informed consent until the last study-related activity. At regular intervals during the study, subjects will be asked non-leading questions to determine the occurrence of any AEs. All AEs reported spontaneously during the course of the study will be recorded as well.

Clinical Laboratory Tests

Blood samples will be collected by venipuncture or via indwelling cannula. Biochemistry and haematology testing will be performed on these samples, as well as immunology testing (hepatitis B surface antigen [HbsAg], anti-hepatitis C virus [HCV] antibody and human immunodeficiency virus [HIV] antibody and antigen) on the sample from screening. In all female subjects, also serum β-HCG assessment at screening and urine β-HCG assessments on Day-1 and at the EOS visit will be performed. Follicle stimulating hormone (FSH) will be measured at screening in all women.

All blood samples for safety assessments, except the ones taken during rescue treatment for Troponin I measurements only, should be taken in a fasted state (overnight fast for at least 8 hours for unbiased glucose determination).

Standard laboratory tests will be performed by ZNA Middelheim.

The following biochemistry and haematology tests will be performed on the safety blood samples:

- Biochemistry: sodium, potassium, chloride, bicarbonate, urate, inorganic phosphate, creatinine, albumin, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamylaminotransferase (GGT), lactate dehydrogenase (LDH), total and direct bilirubin, total serum proteins, blood urea nitrogen (BUN), C reactive protein (CRP), creatine phosphokinase (CPK) and troponin I (Cohorts 2 and 3 only);
- Haematology: haemoglobin, haematocrit, red blood cell (RBC) count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count, platelet count, reticulocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes;
- Coagulation: prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT).

Serum folate will be measured at scheduled time points.

G6PD enzyme test will be performed at screening.

A midstream urine sample will be collected for urinalysis by dipstick for glucose, protein, nitrite, pH and occult blood. Microscopic examination for WBC, RBC and casts will be performed

A urine drug screen (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates) and an alcohol breath test will be performed at scheduled time points.

The Investigator must review the laboratory report, document this review and record any change occurring during the study he/she considers to be clinically relevant in the eSource system. Laboratory values outside the normal range will be flagged and their clinical relevance will be assessed by the Investigator.

Samples that remain after protocol-specific assessments have been performed may be used for further exploratory work on pharmacokinetics, metabolites, plasma protein binding, protein analysis and biochemistry. No human DNA or RNA analysis will be performed.

Vital Signs

Vital sign parameters will be assessed after 5 minutes in supine position at scheduled time points. The vital sign parameters that will be assessed are supine systolic and diastolic blood pressure (SBP and DBP, respectively), pulse rate and body temperature (sublingual). In Cohort 1, orthostatic changes to BP and pulse rate will also be assessed at screening: Subjects will be requested to stand after completion of the supine measurements and blood pressure and pulse rate will be recorded after 2 minutes in the standing position.

These parameters will be measured using a completely automated device consisting of an inflatable cuff and an oscillatory detection system. All values will be registered on a built-in recorder so that measurements are observer-independent.

Any change from baseline in vital sign values occurring during the study that is considered to be clinically relevant by the Investigator should be recorded.

Electrocardiogram

Twelve-lead ECGs recordings will be will be recorded after 10 minutes in supine position at scheduled time points.

All recordings will be performed once, except at screening and before the first and second IMP administration when they will be performed in triplicates at approximately 1-minute intervals. Paper speed will be 25 mm/s, so that the different ECG intervals can be measured manually.

Between Day 10 and 28, one record will be performed on the first day of rescue treatment only.

The interpretations of the ECGs will be performed by the Investigator or his/her designee at the clinical unit. Any change from baseline ECG occurring during the study that is considered to be clinically relevant by the Investigator should be recorded.

Physical Examination

Physical examination will be performed at scheduled time points.

Height is to be measured barefoot and at screening only. Body weight to be measured at scheduled time points. To obtain the actual body weight, subjects must be weighed lightly clothed at screening.

Any change in physical examination occurring during the study that is considered to be clinically relevant by the Investigator should be recorded.

After screening, a targeted, symptom-driven physical examination will be performed focused on changes since the previous examination, but will always include at least: general appearance, skin, heart/circulation, chest, lungs, abdomen and brief neurological examination.

Beck Depression Inventory

The Beck depression inventory is performed at screening only.

The questionnaire is scored by the subject. The inventory completed by the subject will be reviewed/checked for completeness and the total score calculated by the study personnel.

Statistical Methods:

All statistical analysis will be performed by SGS Life Sciences, using SAS® (SAS Institute Inc., Cary, NC, USA; version 9.4 or higher) software for statistical computations.

The standard descriptive statistics for continuous variables are the number of subjects (N), mean, standard deviation (SD), median, first and third quartiles, minimum and maximum values. The standard descriptive statistics for categorical variables are the number of subjects in the category and the proportion expressed as a percentage.

Fasting placebo data from the Cohorts 1, 2 and 3 will be pooled while IMP treatments per cohort are considered as two different treatment groups.

Sample size

Thirty-two subjects will be enrolled in 3 cohorts of 8, 12 and 12 subjects. In agreement with the Sponsor, additional subjects may be recruited in each cohort, to replace discontinuations for non-safety reasons and achieve cohort sizes of 8 and 12.

This is an exploratory study thus no sample size calculation is performed. However, if the nine treated subjects each in Cohort 2 and 3 do not develop positive parasitaemia (qPCR $\geq$ 250 asexual parasites per mL) after IMP administration and until Day 28, it may be concluded that the protection rate for P218 is 0.72 (72%) with a 95% probability (lower limit of exact, one sided test, 95%, CI 0.72). This holds true for both Cohort 2 and Cohort 3.

Planned Analyses

The primary objective of this analysis is the assessment of the chemoprotective activity of P218 in P. falciparum CHMI in non-immune healthy volunteers after PfSPZ Challenge through DVI.

No interim analysis will be performed.

All statistical methods shall be detailed in a Statistical Analysis Plan (SAP) that will be finalized before database lock.

Analysis Populations

The following populations will be considered for analysis:

- Intent-to-treat (ITT) population, defined as all randomised subjects who received at least one dose of IMP and who received the PfSPZ challenge inoculation, analysed as randomised;
- Safety (SAF) population, defined as all subjects who received at least one dose of IMP, analysed as treated;
- Pharmacokinetic (PK) population, defined as all subjects who have received at least 1 dose of IMP and had measurable concentrations of parent and/or metabolites;
- Efficacy population, defined as all subjects in the safety population who received PfSPZ Challenge by DVI;
- PK/PD population, defined as all subjects belonging to both the PK and the efficacy population.

The ITT population will be used for the analysis of demographics and efficacy, the pharmacokinetic population will be used for the pharmacokinetic statistical analysis, the efficacy population for the efficacy statistical analysis and the safety population will be used for safety/tolerability analysis.

Initial Characteristics Data of the Subject Sample

For all randomised and treated subjects, descriptive statistics will be provided per treatment group for demographic (e.g. age, height, weight, BMI, race, gender) and other initial subject characteristics (alcohol and drug screening tests, pregnancy test, orthostatic changes to blood pressure and pulse rate [Cohort 1 only], G6PD enzyme test, serology, medical and social history, concomitant diseases, Beck Depression Inventory).

Prior and concomitant medications will be coded using the World Health Organization (WHO)_DRUG Dictionary.

Pharmacokinetic Data

Pharmacokinetic concentrations will be summarized by treatment group, day and scheduled sampling times by using number of subjects with data, arithmetic mean, standard deviation, coefficient of variation, median, minimum and maximum.

For PK parameters, descriptive statistics will be included with in addition geometric mean and geometric coefficient of variance (CV%); t_{max} will be summarized by using number of subjects with data, median, minimum and maximum.

Individual and mean concentrations versus time figures will be presented.

Efficacy Data

Parasitaemia

Primary endpoint: The duration from PfSPZ challenge DVI to positive parasitaemia will be analysed using descriptive statistics, including the geometric mean and corresponding two-sided 90% confidence intervals. In the absence of positive parasitaemia, the duration will be set to a maximum of 28 days.

The number and proportion of subjects with presence of positive parasitaemia between inoculation with PfSPZ and Day 28 (or the administation of rescue medication) will be summarized by a responder analysis per treatment group. Corresponding two-sided 90% Exact Clopper-Pearson confidence limits will be presented as well.

Malaria Clinical Score

For the malaria clinical score that will be administered by the PI or study physician, actual values and changes from baseline will be evaluated by means of descriptive statistics. Additionally, expected signs and symptoms will be summarized by score.

PK/PD

A model-based analysis is foreseen to characterise the PK/PD relationship between P218 plasma concentrations and blood stage asexual parasitaemia. Details of the modelling analysis will be described in the modelling analysis plan (MAP).

Safety Data

Safety parameters will be tabulated and analysed descriptively.

Adverse Events

The original terms entered in the eSource system by Investigators to identify AEs will be fully described and coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

The reported AEs will be allocated to phases based on their start date. All AEs will be listed. For Cohort 1 all AEs with onset after first IMP treatment will be summarized by treatment group. For Cohorts 2 and 3 all AEs with onset after PfSPZ challenge DVI will be summarised by treatment group. Summaries will be made per MedDRA primary system organ class, MedDRA preferred term, severity, with the number and percentage of subjects and the number of events. Similar summaries will be prepared for AEs considered to be related to IMP and AEs considered to be related to the PfSPZ challenge DVI, for serious AEs and AEs of special interest.

Special attention will be paid to those subjects who died, discontinued IMP due to an AE, or experienced a severe or serious AE. Summaries, listings and narratives may be provided, as appropriate.

Clinical Laboratory Tests

Serum folate, continuous biochemistry and hematology laboratory tests will be evaluated by means of descriptive statistics on the actual values, at each assessment time point and by treatment group. Changes from baseline will also be summarized using descriptive statistics by assessment time point and by treatment group.

Relative changes in clinical laboratory test values compared to values at baseline will be evaluated in accordance with the normal ranges of the clinical laboratory (below, within or above normal range). The percentage of subjects with clinical laboratory test abnormalities will be summarized by treatment group.

The number and percentage of subjects with liver enzyme elevations after IMP administration as defined below will be summarised:

- ALT or AST >3 x Upper Limit of Normal (ULN);
- ALT or AST >5 x ULN;
- ALT or AST >8 x ULN;
- ALT or AST >3 x ULN and bilirubin >2 x ULN at the same time point, together with a conjugated bilirubin fraction> 35% (Potential Hy's law cases).

A listing of subjects with any clinical laboratory test result outside the reference ranges will be provided.

Vital Signs

Vital signs parameters will be assessed after 5 minutes in supine position at scheduled time points. Pulse rate, systolic blood pressure, diastolic blood pressure and body temperature will be evaluated by means of descriptive statistics (actual values and changes from baseline).

The percentage of subjects with vital signs abnormalities will be summarized by treatment group in a cross-tabulation of post-baseline versus baseline abnormalities to the normal ranges.

Electrocardiogram

12-lead ECG recordings will be performed after subjects remained in a supine position for at least 10 minutes. All recordings will be performed once, except at the screening and before the first and second IMP administration when they will be performed in triplicates.

All ECG data automatically measured by ECG devices (PR, QRS, QT, QTcB, QTcF and HR) and overall ECG evaluation will be listed. The ECG data, along with changes from baseline will be summarised by means of descriptive statistics at each assessment time point and by treatment group.

The percentage of subjects with ECG abnormalities will be summarized by treatment group in a cross-tabulation of post-baseline versus baseline abnormalities to the normal ranges.This cross-tabulation will include categorical assessment on actual values and changes from baseline of QTcB and QTcF prolongation.

Physical Examination

Abnormal findings in physical examination will be listed.

TIME AND EVENTS SCHEDULE

Cohort 1

Description	SCR	Treatment and follow-up																							EOS
Study Day	-28 to -2	-1[a]	1						2		3								4		5	6	7	8	9
Timepoint (h) in relation to 1st IMP administration		0	0[b]	1	2[b]	4	6	10	24	36	48	48.5	49	49.5	50	52	54	58	72	84	96[c]	120[c]	144[c]	168[c]	192[c]
Ambulatory visit	X																				X	X	X	X	X
Confinement in clinical unit[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Eligibility criteria	X	X																							
Informed consent[o]	X																								
Demographics	X																								
Medical and social history	X																								
Beck depression inventory	X																								
Randomisation[p]			X																						
Alcohol & drug screen[e]	X	X																							
Height & weight[f]	X	X																		X					X
Physical examination[g]	X	X							X										X						X
Vital signs[h]	X	X	X	X	X			X	X		X				X			X	X	X		X			X
12-lead ECG[i]	X	X	X[i]						X		X[i]								X			X			X
Serology[j]	X																								
Pregnancy test[k]	X	X																							X
IMP administration[l]			X								X														
Haematology, chemistry & urinalysis[q]	X	X									X											X			X
Serum folate	X	X[r]									X											X			X
Coagulation parameters[m]	X	X									X											X			X
PK blood sample[n]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[n]	X	X	X	X	X
Previous medications	X																								

Description	**SCR**	**Treatment and follow-up**																						**EOS**
Study Day	**-28 to -2**	**-1[a]**	**1**						**2**		**3**								**4**		**5**	**6**	**7**	**8**	**9**
Timepoint (h) in relation to 1st IMP administration		**0**	**0[b]**	**1**	**2[b]**	**4**	**6**	**10**	**24**	**36**	**48**	**48.5**	**49**	**49.5**	**50**	**52**	**54**	**58**	**72**	**84**	**96[c]**	**120[c]**	**144[c]**	**168[c]**	**192[c]**
Concomitant medications		X----------X																						
AEs		X----------X																						
SAE reporting	X----------X																							

AE: adverse event, ECG: electrocardiogram, EOS: end of study, IMP: investigational medicinal product, PK: pharmacokinetic, SAE: serious adverse event, SCR: screening

a. Assessments begin as of subject confinement in the clinical unit and must be completed and outcomes must be available before IMP administration.
b. The assessments indicated must be completed and outcomes must be available before IMP administration. Predose PK sample should be taken within 1 hour before IMP administration.
c. The assessments indicated will be performed daily during ambulatory visits to the clinical unit.
d. Subjects will be admitted to the clinical unit in the morning of Day -1 and will remain in confinement until Day 4, i.e. 36 hours after second IMP administration.
e. Alcohol breath test and urine dipstick screening for drug abuse.
f. Height to be measured at screening only; body weight to be measured at screening, upon admission to the clinical unit on Day-1, upon discharge from the clinical unit on Day 4 and at the EOS visit on Day 9.
g. Full physical examination will be conducted at screening and at the EOS visit on Day 9. Targeted (symptom-driven) physical examination will be conducted on all other occasions. Symptom-driven physical examination will be conducted at any time during follow-up, if indicated.
h. Vital signs (blood pressure, pulse and body temperature) will be measured after remaining 5 minutes in a supine position. At screening, orthostatic changes to blood pressure and pulse rate will also be assessed: subjects will be requested to stand after completion of the supine measurements and blood pressure and pulse rate will be recorded after 2 min in the standing position.
i. 12-lead ECGs recordings will be performed after subjects have remained in a supine position for at least 10 minutes. All recordings will be performed once, except at the screening and before the first and second IMP administration when they will be performed in triplicates.
j. Serological testing for human immunodeficiency virus (HIV) antibody and antigen, hepatitis B surface antigen (HbsAg) and anti-hepatitis C virus (HCV) antibody, to determine eligibility for the trial.
k. Pregnancy testing consists of serum β-human chorionic gonadotropin (β-HCG) assessment at screening and urine β-HCG assessments on Day-1 and at the EOS visit.
l. In each instance, the subject will receive a single oral dose of 1000 mg P218 or placebo after fasting at least 8 hours (see Section 4.3).
m. International Normalised Ratio (INR), prothrombin time (PT) and activated partial thromboplastin time (aPTT).
n. On Day 4, the subject will be discharged from the clinical unit only after the PK sample at 84 hours is drawn.
o. No study-related procedure is to be performed before voluntarily signing of the informed consent form.
p. Randomisation will be performed before first IMP administration.
q. Laboratory tests will be performed after fasting for at least 8 hours.
r. This assessment can be done at either D-3 or D-1, upon Investigator's decision.

Cohorts 2 and 3

Description	SCR	Challenge, treatment and follow-up																																EOS visit	EOS [u]
Study Day	**-28 to -2**	**-1[a]**	**1**									**2**		**3**								**4**		**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14-28[c]**	**35**	**45 max**
Timepoint (h) in relation to PfSPZ Challenge		**0**	**0[b]**	**2[b]**	**2.5**	**3**	**3.5**	**4**	**6**	**8**	**12**	**24**	**36**	**50**	**50.5**	**51**	**51.5**	**52**	**54**	**56**	**60**	**72**	**84**	**96**	**120**	**144**	**168**	**192**	**216**	**240**	**264**	**288**			
Ambulatory visit	X																																X	X	
Confinement in clinical unit[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Eligibility criteria	X	X																																	
Informed consent[e]	X																																		
Demographics	X																																		
Medical and social history	X																																		
Beck depression inventory	X																																		
Randomisation[t]				X																															
Alcohol & drug screen[f]	X	X																																	
Height & weight[g]	X	X																														X		X	
Physical examination[h]	X	X										X										X		X[h]			X		X[h]			X	X[h]	X	
Vital signs[i]	X	X	X	X				X			X	X		X				X	X		X	X	X	X			X	X	X	X	X	X	X	X[i]	X	
12-lead ECG[j]	X	X	X[j]	X[j]				X			X	X		X[j]				X	X		X		X											X[j]	X	
G6PD deficiency enzyme test	X																																		
Serology[k]	X																																		
Pregnancy test[l]	X	X																																X	

Description	SCR	Challenge, treatment and follow-up																																EOS visit	EOS [u]
Study Day	**-28 to -2**	**-1[a]**	**1**									**2**		**3**								**4**		**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14-28[c]**	**35**	**45 max**
Timepoint (h) in relation to PfSPZ Challenge		**0**	**0[b]**	**2[b]**	**2.5**	**3**	**3.5**	**4**	**6**	**8**	**12**	**24**	**36**	**50**	**50.5**	**51**	**51.5**	**52**	**54**	**56**	**60**	**72**	**84**	**96**	**120**	**144**	**168**	**192**	**216**	**240**	**264**	**288**			
DVI of PfSPZ Challenge			X																																
IMP administration[m]				X										X																					
Malaria clinical score[n]			X																							X	X	X	X	X	X	X	X	X	
Haematology, chemistry & urinalysis[o]	X	X												X													X		X				X[o]	X	
Serum folate	X	X[v]												X													X		X				X[o]	X	
Coagulation parameters[p]	X	X												X													X								
PK blood sample			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							
Rescue therapy[q]																																		X	
TBS[r]																										X	X	X	X	X	X	X	X	X	
qPCR for parasites[s]			X																							X	X	X	X	X	X	X	X	X	
Previous medications	X																																		
Concomitant medications		X----------																																----X	
AEs		X----------																																----X	
SAE reporting	X----------																																	----X	

AE: adverse event, DVI: direct venous inoculation, ECG: electrocardiogram, EOS: end of study, G6PD: glucose-6-phosphate dehydrogenase, IMP: investigational medicinal product, PfSPZ: *Plasmodium falciparum* sporozoites, PK: pharmacokinetic, qPCR: quantitative polymerase chain reaction, SAE: serious adverse event, SCR: screening, TBS: thick blood smear

a. Assessments begin as of subject confinement in the clinical unit and must be completed and safety assessment outcomes must be available before PfSPZ Challenge DVI on Day 1.
b. On Day 1, assessments begin as of 3 hours before challenge. The assessments indicated must be completed and safety assessment outcomes must be available before PfSPZ inoculation or before IMP administration. Predose PK sample should be taken within 1 hour before IMP administration.
c. The assessments indicated will be performed daily during ambulatory visits to the clinical unit.
d. Subjects will be admitted to the clinical unit in the morning of Day -1 and will remain in confinement until Day 13, i.e. 12 days post PfSPZ Challenge.
e. No study-related procedure is to be performed before voluntarily signing of the informed consent form.
f. Alcohol breath test and urine dipstick screening for drug abuse.
g. Height to be measured at screening only; body weight to be measured at screening, upon admission to the clinical unit on Day-1, upon discharge from the clinical unit on Day 13 and at the EOS visit on Day 35.

h. Full physical examination will be conducted at screening, at Days -1, 2, 4, 8 and 13, and at the EOS visit on Day 35. Targeted (symptom-driven) physical examination will be conducted on Days 5, 10, 15, 20 and 25, and at the start of rescue treatment. Symptom-driven physical examination will be conducted at any time during follow-up, if indicated.
i. Vital signs (blood pressure, pulse and body temperature) will be measured after remaining 5 minutes in a supine position. During the period between Day 14 and 28, vital signs will be measured on Days 15, 20 and 25 and on the first day of rescue treatment only.
j. 12-lead ECGs recordings will be performed after subjects have remained in a supine position for at least 10 minutes. All recordings will be performed once, except at the screening and before the first and second IMP administration when they will be performed in triplicates. During the period between Day 14 and 28, one record will be performed on the first day of rescue treatment only.
k. Serological testing for HIV antibody and antigen, HbsAg and anti-HCV antibody, to determine eligibility for the trial.
l. Pregnancy testing consists of serum β-HCG assessment at screening and urine β-HCG assessments on Day-1 and at the EOS visit.
m. In each instance, the subject will receive a single oral dose of 1000 mg P218 or placebo (Cohort 2) or a single oral dose of 100 mg P218 or placebo (Cohort 3) after fasting 8 hours at least (see Section 4.3). The initial dose at Day 1 must not be given prior to 2 hours post-inoculation.
n. Malaria clinical score for malaria signs and symptoms is assessed on Day 1, in the 3 hours before PfSPZ Challenge, and daily from Day 7 to the day subject is positive for parasitaemia and at the EOS visit at Day 35. Daily assessments will take place during rescue treatment. For subjects that not develop positive parasitaemia until Day 28, after Day 28, assessments will take place only at the EOS visit at Day 35.
o. During the period between Day 14 and Day 28, haematology, clinical chemistry and urinalysis laboratory tests will be performed and serum folate measured after fasting for at least 8 hours on Days 14, 21 and 28, and additionally one day after the subject turned positive for parasitaemia, if not coincident with one of these scheduled timepoints. Troponin I will be measured at screening, baseline prior to inoculation and on Days 2 and 3 of rescue treatment (see Section 5.2).
p. INR, PT and aPTT.
q. Every subject will receive a three-day course of antimalarial rescue medication as soon as positive for parasitaemia or on Day 28 in subjects who do not develop positive parasitaemia throughout the follow-up period (see Section 5.2). On the first day of rescue treatment, the subject will also receive a single oral dose of Primaquine® to ensure complete clearance of gametocytes.
r. Blood samples for the assessment of parasitaemia by TBS microscopy will be drawn daily from Day 7 to Day 28. In subjects who develop parasitaemia and receive rescue treatment, TBS microscopy will be performed daily for confirmation of the qPCR outcomes until treatment completion and success (see Section 5.2 for definition), and at the EOS visit on Day 35. In subjects who do not develop positive parasitaemia until Day 28 of the study, rescue treatment will be initiated on Day 28. Instead of daily qPCR and TBS assessments until treatment success, for these subjects only a final qPCR will be performed at the EOS visit on Day 35.
s. Blood samples for the assessment of parasitaemia by qPCR will be drawn at the following timepoints: immediately before PfSPZ Challenge DVI and daily from Day 7 to Day 28. In subjects who develop parasitaemia and receive rescue treatment, qPCR will be performed daily until treatment completion and one negative outcome is obtained, and at the EOS visit on Day 35. In subjects who do not develop positive parasitaemia until Day 28 of the study, rescue treatment will be initiated on Day 28. Instead of daily qPCR and TBS assessments until treatment completion and success, for these subjects only a final qPCR will be performed at the EOS visit on Day 35.
t. Randomisation will be performed before first IMP administration.
u. All subjects that receive rescue therapy will be asked non leading questions to determine the occurrence of any AEs approximately two weeks after initiation of the rescue treatment, either during a planned study visit or by phone in case there is no planned study visit at that time. If this time point is after the EOS visit on Day 35, the moment of the phone call will be considered the EOS and not Day 35.
v. This assessment can be done at either D-3 or D-1, upon Investigator's decision.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

List of Abbreviations

β-HCG	β-human chorionic gonadotropin
λ_z	First order terminal rate constant
ABV	alcohol by volume
AE	Adverse event
AESI	Adverse events of special interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
API	Active pharmaceutical ingredient
aPTT	activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
$AUC_{0\text{-inf}}$	Area under the plasma concentration-time curve from time zero to infinity
$AUC_{0\text{-last}}$	Area under the plasma concentration-time curve from time zero to the last quantifiable concentration
$AUC_{48h\text{-inf}}$	Area under the plasma concentration-time curve from 48 hours after dosing to infinity
$AUC_{48h\text{-last}}$	Area under the plasma concentration-time curve calculated between 48 hours after dosing and last quantifiable concentration
AUC_{τ}	Area under the plasma concentration-time curve during the dosing interval
$AUC_{0\text{-}48h}$	Area under the plasma concentration-time curve during the first 48 hours after dosing
$AUC_{x\text{-}yh}$	Area under the plasma concentration-time curve from time x to y hours post-dose
AUC_{inf}	Area under the plasma concentration-time curve from time zero to infinity
BMI	Body mass index
BSC	Biosafety cabinet
CHMI	Controlled human malaria infection
CL/F	Total plasma clearance
C_{max}	Observed maximum plasma concentration
C_t	Last observed quantifiable concentration
DVI	Direct venous inoculation
DBP	Diastolic blood pressure
DHFR	dihydrofolate reductase
DNA	Deoxyribonucleic acid
DVI	Direct venous injection
ECG	Electrocardiogram
ED_{90}	effective dose for 90% of the animals
EOS	End-of-study
FIH	first-in-human
FSH	Follicle stimulating hormone
G6PD	Glucose-6-phosphate dehydrogenase

GCP	Good Clinical Practice
GI	Gastrointestinal
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HSA	Human serum albumin
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IMP	Investigational medicinal product
INR	international normalized ratio
IRB	Institutional Review Board
ITM	centre of excellence for tropical medicine
ITT	Intent-to-treat
LPLV	Last Patient Last Visit
MCH	Mean corpuscular hemoglobin
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimal inhibitory concentration
MHRA	Medicines and Healthcare products Regulatory Agency
MPC	Minimal parasiticidal concentration
NSAID	non-steroidal anti-inflammatory drug
PBS	Phosphate buffered saline
P. falciparum	*Plasmodium falciparum*
PD	Pharmacodynamic(s)
Pf	*P. falciparum*
PfSPZ	*P. falciparum* sporozoites
PI	Principal Investigator
PK	Pharmacokinetic(s)
PopPK	population pharmacokinetics
PT	Prothrombin time
qPCR	quantitative polymerase chain reaction
QTcB	QT interval corrected according to Bazett's formula
QTcF	QT interval corrected according to Fridericia's formula
R_{ac}	Accumulation ratio
RBC	Red blood cell
RNA	Ribonucleic acid
SAE	Serious adverse event
SBP	Systolic blood pressure
SPZ	Sporozoites
SRT	Safety Review Team
$t_{½}$	Terminal elimination half-life
TBS	Thick blood smear
TEAE	Treatment-emergent adverse event
t_{max}	Time to reach the maximum concentration after drug administration
ULN	Upper Limit of Normal

Vz/F	Apparent volume of distribution
WBC	White blood cell
WHO	World Health Organization

Definitions of Terms

BMI	Weight in kilogram divided by the square of height in meters
QTcB	QT interval corrected according to Bazett's formula: QTcB (ms)=QT (ms)/$RR^{1/2}$ where RR=(60/heart rate)*1000
QTcF	QT interval corrected according to Fridericia's formula: QTcF (ms)=QT (ms)/$RR^{1/3}$ where RR=(60/heart rate)*1000
Positive parasitaemia	Defined as qPCR outcome equal or greater than 250 asexual parasites per mL. The presence of asexual parasites will be confirmed by thick blood smear (TBS) microscopy.

STUDY ADMINISTRATIVE STRUCTURE AND INVESTIGATORS

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1. INTRODUCTION

1.1 MALARIA BACKGROUND INFORMATION

Malaria is the second most prevalent infectious disease in the world and threatens half of the world's human population. In 2016, the World Health Organization (WHO) reported 216 million new cases of malaria worldwide and 445,000 deaths [21]. Children <5 years of age accounted for 70% of deaths in 2015. The majority of these deaths were due to infection with *Plasmodium falciparum* (*P. falciparum*) [20]. Around the world, effective disease control programs relying on artemisinin-containing combination therapies (ACT) such as Coartem® have contributed to a global reduction in the mortality rate of *P. falciparum* malaria. However, recent reports suggest that decades of continuous use of artemisinins as monotherapies may have fostered drug resistance against artemisinin-derivatives (the last widely effective antimalarial drugs).

Malaria is a serious and life-threatening disease and owing to increasing resistance to the current antimalarial therapies, new therapies are required as both stand-alone and partner therapies to address a growing unmet medical need.

1.2 P218

P218 is a selective inhibitor of *Plasmodium* dihydrofolate reductase (DHFR), an enzyme catalysing the reduction of folates to tetrahydrofolates, which are essential for DNA biosynthesis in the malarial parasite [22]. DHFR inhibitors, selective to the *Plasmodium* DHFR not targeting the distinct human DHFR, are a validated target for malaria treatment. Pyrimethamine, another DHFR inhibitor, marketed in combination with sulfadoxine, has been used for malaria treatment for decades. Its usage for malaria treatment was stopped worldwide following emergence and spread of resistance. However, it is still WHO-recommended for prophylactic use solely or in combination as seasonal malaria chemoprevention (SMC) in West Africa and as Intermittent Preventive Treatment during Pregnancy (IPTp) and in Infants (IPTi) in malaria endemic areas where benefits are still expected.

P218 demonstrates activity in vitro and in vivo on pyrimethamine *P. falciparum* resistant strains suggesting that this molecule may offer a favourable treatment advantage over pyrimethamine.

P218 has potential value as chemoprotective agent against *P. falciparum* malaria as it showed strong activity in vitro on *P. yoelii*, *P. berghei* and *P. falciparum* liver sporozoites with a 50% inhibitory concentration (IC_{50}) of 2.14, 2.0 and 0.94 nM, respectively. The P218 in vitro inhibitory activity on *P. falciparum* DHFR was comparable to, or greater than pyrimethamine.

Based on these encouraging data, P218 is primarily considered for malaria chemoprotection.

A first in human (FIH) study was conducted in the UK (MMV_P218_15_01/C16009). It was a double-blind, randomised, placebo-controlled, ascending-dose study with Part A (single ascending doses of 10 mg to 1000 mg) and Part B (food effect assessment). There were no serious adverse events (SAEs) or severe adverse events in subjects who received a single oral dose of up to 1000 mg P218. The highest single dose tested (1000 mg) was

considered safe and well tolerated and was associated with mean maximum observed plasma concentration (C_{max}) and area under the plasma concentration-time curve from time zero to infinity ($AUC_{0\text{-}inf}$) values of 8.64 µg/ml and 17.8 µg·h/ml, respectively. This provides an exposure margin of 4.0-fold to the protocol-defined stopping criteria of 71.9 µg·h/ml based on total cumulative AUC from time 0 to 336 hours post dose ($AUC_{0\text{-}336h}$) observed in the most sensitive species (dog). The single oral dose of 1000 mg of P218 was associated with a mean elimination half-life of 19.6 h which is too short to allow for weekly or monthly oral dosing in a chemoprotection setting.

Antimalarial efficacy against the blood stage *P. falciparum* has also been evaluated in a validated mouse model (Humanized SCID mouse) with established effective dose for 90% of the animals (ED_{90}) of 1.6 mg/kg/day and derived minimal parasiticidal concentration (MPC) and minimal inhibitory concentration (MIC) of 4.4 ng/ml and 1.3 ng/ml, respectively. A human dose that could maintain blood concentrations above MIC was identified as a target minimal efficacious dose for chemoprotection against *P. falciparum* malaria. Using this approach and observed human pharmacokinetics (PK) in the completed FIH study, it is likely that using the currently available oral formulation of P218, a single human dose of 1000 mg would be needed to maintain in human subjects P218 plasma concentrations above the target mouse blood MIC for 48 hours.

During conduct of the FIH study, two acyl glucuronides previously identified in preclinical species were also observed in healthy subjects. Given that the structure and in particular, the site of conjugation are identical between both acyl glucuronides of P218, it is considered appropriate to assess safety in terms of total acyl glucuronide burden rather than each one independently [19]. In agreement with the Medicines and Healthcare products Regulatory Agency (MHRA), toxicology coverage of human P218 β-acyl glucuronide and P218-OH β-acyl glucuronide was therefore assessed by considering both molecules as a whole, with total human AUC being the sum of human AUC of each molecule. The observed P218 β-acyl glucuronides total human $AUC_{0\text{-}inf}$ at the 1000 mg dose was 54.4 µg·h/ml which remains below the highest tolerated, estimated cumulative glucuronides AUCs in the 14-day Good Laboratory Practice (GLP) toxicology study in dog (74.6 µg·h/ml) and the study in rat (62.5 µg·h/ml).

Please refer to the current Investigator's Brochure (IB) for more detailed information on P218 [6].

1.3 *PLASMODIUM FALCIPARUM* SPOROZOITES CHALLENGE MODEL (PFSPZ CHALLENGE)

P. falciparum sporozoites challenge model (PfSPZ Challenge) consists of aseptic, purified, live, infectious, cryopreserved *P. falciparum* (Pf) sporozoites (SPZ) (PfSPZ Challenge) obtained from *Anopheles stephensi* mosquitoes reared under aseptic conditions and infected with *P. falciparum* sporozoites of either the NF54 strain or 7G8 clone. The PfSPZ Challenge product was developed to infect volunteers by controlled human malaria infection (CHMI) to assess the efficacy of antimalarial drugs and vaccines and the effects of naturally acquired immunity and innate resistance. PfSPZ Challenge is administered by trained clinical study staff with a needle and syringe as directed by the protocol.

Malaria Challenge has been safely used in multiple clinical trials in the United States, Europe, Australia and Africa via the intradermal (ID), intravenous (IV) and intramuscular (IM) routes [4, 5, 9, 10, 12, 15, 16, 17, 18]. As of 17 April 2018, 913 volunteers have received 1407 doses of PfSPZ Challenge (NF54) and 34 volunteers have received 34 doses of PfSPZ Challenge (7G8). Extensive safety and efficacy data are available [7]. In addition, PfSPZ Challenge has been safely administered to volunteers in combination with antimalarial drugs. Currently, the validated inoculation method for drug and vaccine evaluation consists of direct venous injection (DVI) with an inoculum size of 3,200 sporozoites which has been shown to induce subclinical malaria in 100% of inoculated healthy volunteers [11].

Please refer to the current IB for more detailed information on the PfSPZ Challenge [7].

1.4 RATIONALE FOR THE STUDY

The present study will investigate P218 as possible chemoprotective agent against *P falciparum* in a standardised and validated CHMI model using DVI of aseptic, purified, cryopreserved, vialed *P. falciparum* sporozoites (PfSPZ Challenge).

If the P218 activity against *P. falciparum* infection is clinically demonstrated in this study, a long acting injectable depot formulation will be considered, allowing for slow-blood-release of P218 over at least one week and preferably, over one month following single injection [1].

1.5 RISK BENEFIT ANALYSIS

1.5.1 *Potential Risks*

To date, P218 has been administered as a single dose to 50 healthy human subjects. The pharmacokinetic, safety and tolerability data from these subjects suggest a favourable tolerability and safety profile. This is supported by the pharmacological and toxicological profile of P218 observed in preclinical studies.

Potential Risks Based on Non-Clinical Studies

The gastrointestinal (GI) toxicity (erosion, epithelial hyperplasia, inflammation and a mucosal inflammatory infiltrate of the caecum) observed in nonclinical studies (14-day repeated dose in rats and dogs) was largely reversible after 14 days off-dose, although slight ulceration, epithelial hyperplasia, dilated glands, inflammation and a mucosal inflammatory infiltrate of the caecum persisted in female animals.

Co-administration of folate in rats completely abolished the histopathological effects observed on the GI tract. The mechanism for the GI toxicity has been established to be caused by folate depletion and is consistent with the pathology observed after treatment with other DHFR inhibitors. Based on reported GI toxicity in clinical trials conducted with pyrimethamine, a marketed DHFR inhibitor for malaria, the following adverse events could be reported in human subjects after P218 administration: anorexia, nausea, vomiting and less commonly leucopoenia, thrombocytopenia and megaloblastic anaemia (due to folate depletion).

Gall bladder pathology (epithelial dysplasia and/or apoptosis, and inflammation) was observed in 14-day repeated dose study in dogs. These microscopic findings were found to be reversible in males and partially reversible in females.

Administration of P218 to rats or dogs results in exposure to all human metabolites. Exposure to P218-OH is quantitatively lower in humans than the 10% threshold accepted under Metabolites in Safety Testing (MIST) guidance to require further evaluation and is therefore considered qualified.

Two acyl glucuronides have been identified to be present in all species but quantitative differences between the species used in toxicology and humans have been demonstrated. Given that the structure and in particular the site of conjugation is identical between both acyl glucuronides of P218 and in agreement with the MHRA, it is considered appropriate to assess safety in terms of total acyl glucuronide burden rather than considering each one independently [19]. This approach allows compensating partly for the likely high variation in specific acyl glucuronides of parent drug and metabolites across species where variation across Phase 1 and Phase 2 metabolite enzymes will be magnified. These include oxidative and uridine 5'-diphospho-glucuronic transferases (UDPGTs) responsible for formation of glucuronides, but also hydrolytic enzymes (esterases) which will influence degradation.

The toxicology coverage of human P218 β acyl glucuronide and P218-OH β acyl glucuronide was therefore assessed by considering both molecules as a whole, with the global human AUC from time zero to the last quantifiable concentration ($AUC_{0\text{-last}}$) being the sum of the human $AUC_{0\text{-last}}$ of each molecule. Under this assessment all metabolites are considered qualified as exposure in the toxicology species was higher than the exposures observed or expected in humans. Importantly no toxicity that was not related to DHFR inhibition was observed in either species, supporting the conclusion that none of the metabolites pose an additional toxicological risk. For more information, please refer to the IB [6].

Potential Risks Based on Clinical Data

To date P218 was administered in one Phase 1 study performed in the UK. The FIH study (MMV_P218_15_01) included a total of 64 adult male and female (women of non-childbearing potential [WONCBP]) healthy subjects. In Part A, 42 subjects received P218 at singles doses of 10 mg (n=6), 30 mg (n=6), 100 mg (n=6), 250 mg (n=6), 500 mg (n=6), 750 mg (n=6) or 1000 mg (n=6) and 14 subjects received placebo (two each per dose level). In Part B, four subjects received a single dose of 250 mg P218 when fasted in Period 1 and fed in Period 2, four other subjects received 250 mg P218 when fed in Period 1 and fasted in Period 2. Subjects were administered P218 as a capsule for oral administration.

Overall, in this clinical study, there were no significant abnormalities on laboratory parameters including hepatic transaminases, vital signs, electrocardiograms (ECGs) or physical examination and there were only five drug related treatment-emergent adverse events (TEAEs) in Part A (and none in any fasted subjects in Part B). These findings indicate that P218 was safe and well tolerated when administered, fasted, in single doses of up to 1000 mg. There did not appear to be any effect on the tolerability profile of P218 when administered as a single dose in the fed state on the basis of this study.

P218 was rapidly absorbed with C_{max} values achieved between 30 minutes and 2 hours post dose. Plasma concentrations of P218 decline in a bi-exponential manner with P218 half-life values ranging from 3.1 to 6.7 hours at the two lowest doses (10 and 30 mg) and increase up to 8.9-19.6 hours at doses up to 1000 mg. P218 parent and metabolite exposure values as assessed by C_{max} and $AUC_{0\text{-}inf}$, increase in a dose-proportional manner between 100 and 1000 mg. Co-administration of P218 with food reduced C_{max} (35%) and delayed absorption (1 hour) but with no significant impact on AUC.

Potential Risks Associated With PfSPZ Challenge

Subjects receiving the PfSPZ DVI will be exposed to additional risks, including the development of malaria symptoms as well as adverse effects of the approved rescue medication (Riamet® or Malarone® and Primaquin®) to be prescribed as curative therapy for parasite clearance prior to the end of the study.

The CHMI PfSPZ challenge model with the DVI technique is a well-validated method for obtaining informative data on the clinical activity of novel antimalarial compounds. It has replaced the traditional method of CHMI by the bite of mosquitos to measure vaccine and drug efficacy. In a series of clinical trials, inoculation of PfSPZ Challenge was found to be safe and well tolerated with the optimal dose established as 3200 PfSPZ administered by DVI.

Risks to subjects in this Phase IB study will be minimized in the following ways:

- Sentinel dosing of subjects in Cohort 1 (Please refer to Section 3.1);
- Progression to consecutive study cohorts only after evaluation of data from previous cohorts and approval from Safety Review Team (SRT) (Please refer to Section 5.8);
- Adherence to the inclusion/exclusion criteria: only subjects considered suitable according to these criteria and who are not at any perceived risk will be included. Subjects with gallbladder disease (e.g. cholecystitis, cholelithiasis, cholecystectomy), or megaloblastic anaemia secondary to folate deficiency will not be included;
- The dose of PfSPZ inoculum and route of administration has previously been shown to be safe and well-tolerated in healthy human subjects;
- Regular and intense monitoring to ensure the safety and well-being of the study subjects will include the following:
    - Admission to the clinical unit in the morning of Day -1, i.e. from at least 8 hours before first investigational medicinal product (IMP) administration or for Cohorts 2 and 3 before PfSPZ Challenge until 12 days post PfSPZ Challenge;
    - Vital signs (blood pressure, heart rate, body temperature);
    - 12-lead ECGs;
    - Safety laboratory assessments of clinical chemistry and haematological blood parameters performed at scheduled time points and repeated, if necessary, to ensure appropriate follow-up of any clinically relevant abnormality;
    - Daily parasitaemia assessments by both quantitative polymerase chain reaction (qPCR) and Thick Blood Smear (TBS) techniques from Day 7

post inoculation until the subject is positive for parasitaemia or until follow-up to Day 35 post inoculation is completed;
    - Objective/directed evaluation of Malaria signs and symptoms through a clinical score.
- All subjects in Cohorts 2 and 3 will be prescribed curative therapy for malaria for radical clearance during or at the end of the study;
- The total volume of blood drawn from each subject will not exceed 450 ml in any given 30-day period. This volume includes allowance for unscheduled safety laboratory assessments that may be required at the discretion of the Principal Investigator (PI) or MMV to ensure subjects safety;
- In addition to the PI or his representative, the site has access to an expert medical malariologist to help in making clinical decisions as necessary, including in the rare event that hospitalization is required, which will be done at the Antwerp University Hospital (UZA). SAEs will be treated according to their nature at a specialist inpatient facility.

1.5.2 *Potential Benefits*

P218 will be given to healthy subjects purely for research and development purposes and those subjects receiving P218 will experience no medical benefit except for a general health examination.

Benefits of the study are society-based and related to possible future antimalarial therapies. Taking into consideration the proposed risk-management plan as described, the risk to subjects participating in this study is considered to be minimal and acceptable.

1.5.3 *Conclusion*

No benefit is expected for individual subjects participating in this study. Benefits of the study are society-based and related to possible future antimalarial therapies. Taking into consideration the proposed risk-management plan as described, the risk to subjects participating in this study is considered to be minimal and acceptable.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 OBJECTIVES

2.1.1 *Primary Objective*

2.1.1.1 *COHORT 1*

The primary objective of this cohort is to assess the safety and tolerability of two single doses of 1000 mg P218 administered 48 hours apart in healthy adult volunteers.

2.1.1.2 *COHORTS 2 AND 3*

The primary objective of these cohorts is to assess the chemoprotective activity of P218 in *P. falciparum* CHMI in non-immune healthy volunteers after PfSPZ Challenge through DVI.

2.1.2 *Secondary Objectives*

Secondary objectives are:

- To assess the PK profile of P218, and in Cohort 1 only also of its main metabolites, up to 8 days after the first P218 administration to healthy adult volunteers;
- To establish the pharmacokinetic/pharmacodynamic (PK/PD) relationship between P218 plasma concentration and its chemoprotective activity using blood stage parasitaemia as a surrogate in non-immune healthy volunteers in a CHMI PfSPZ Challenge model;
- To assess the safety and tolerability of P218 in non-immune healthy volunteers in a CHMI PfSPZ Challenge model (Cohorts 2 and 3);
- To assess the safety and tolerability of PfSPZ Challenge in non-immune healthy volunteers before and after P218 administration during CHMI (Cohorts 2 and 3).

There is no formal hypothesis testing as this is an exploratory study.

2.2 ENDPOINTS

2.2.1 *Primary Endpoints*

2.2.1.1 *COHORT 1*

The primary endpoint of this cohort is the incidence, severity and relationship to P218 of observed or self-reported TEAEs during the 9-day observation period without PfSPZ Challenge.

2.2.1.2 *COHORTS 2 AND 3*

The primary endpoint of these cohorts is the cohort-specific, geometric mean time to parasitaemia based in each case on the time elapsed between PfSPZ Challenge DVI and first qPCR outcome equal or greater than 250 asexual parasites per mL, with a maximum of 28 days in the absence of parasitaemia.

2.2.2 *Secondary Endpoints*

Secondary endpoints are:

- Estimation of the following PK parameters for P218, and in Cohort 1 only, also for its major metabolites (P218-OH, P218-beta-acyl-glucuronide, P218-beta-acyl-glucuronide-OH), over 8 days after first IMP administration using non-compartmental methods:
    - Area under the plasma concentration-time curve during the dosing interval (AUC_{τ}) = AUC during the first 48 hours after dosing ($AUC_{0\text{-}48h}$) after each administration (i.e. $AUC_{0\text{-}48h}$ and $AUC_{48\text{-}96h}$);
    - AUC from 48 hours after dosing to infinity ($AUC_{48h\text{-}inf}$), calculated from $AUC_{48h\text{-}t} + (Ct/\lambda z)$, where C_t is the last observed quantifiable concentration and λ_z the first order terminal rate constant;
    - AUC calculated between 48 hours after dosing and last quantifiable concentration ($AUC_{48h\text{-}last}$);
    - Total plasma clearance (CL/F) and apparent volume of distribution (Vz/F) after the second administration (not for metabolites);
    - C_{max} and the time to reach the maximum concentration after drug administration (t_{max}) after each administration;
    - The terminal elimination half-life ($t_{1/2}$) after the second administration;
    - The accumulation ratio (R_{ac}), calculated as $AUC_{48\text{-}96h}/AUC_{0\text{-}48h}$;
    - Metabolic ratio of AUC_{τ} and $AUC_{48h\text{-}inf}$ for P218 β-acyl glucuronide, P218-OH and P218-OH β-acyl glucuronide over parent P218 (Cohort 1 only).
- The minimal inhibitory concentration of P218 needed for chemoprotective activity will be predicted from the P218 PK/PD model;
- The incidence, severity and relationship to P218 of observed or self-reported, TEAEs during the 35-day observation period with PfSPZ Challenge (Cohorts 2 and 3 only);
- The incidence and severity of observed (i.e. Malaria signs and symptoms graded by the Malaria clinical score; see Attachment 1) or self-reported inoculum-related events and adverse events (AEs) considered PfSPZ Challenge-related (Cohorts 2 and 3 only);
- The Malaria clinical score at the time of introduction of rescue therapy (Cohorts 2 and 3 only);
- Changes from baseline in folate levels, haematology, clinical chemistry and urinalysis parameters, vital signs and ECG parameters.

3. STUDY DESIGN

3.1 OVERVIEW OF STUDY DESIGN

This is a single centre, randomised, double-blind, placebo-controlled Phase Ib study. Thirty-two healthy men and women aged 18 to 45 years will be enrolled in 3 cohorts of 8, 12 and 12 subjects. A subject may be enrolled in one cohort only and will be randomised in a 3:1 ratio, to receive two consecutive administrations of either P218 or placebo. Enrolment in cohorts will proceed sequentially, to facilitate review of Cohort 1 and Cohort 2 data by an SRT before populating Cohort 2 and Cohort 3, respectively.

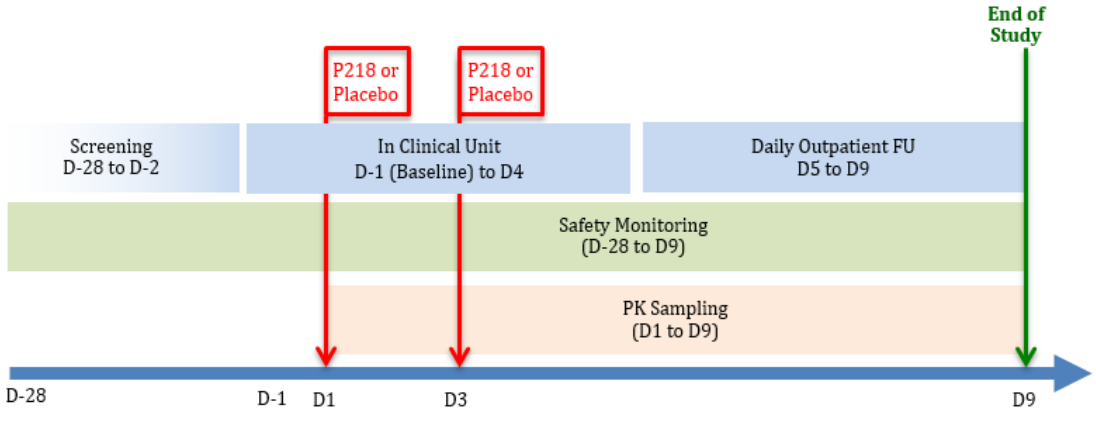
Cohort 1

Cohort 1 will consist of 2 subgroups of subjects, to be enrolled sequentially: subgroup 1 will be composed of 2 subjects: one to receive two 1000 mg doses of P218 48 hours apart and one to receive placebo; subgroup 2 will be composed of 6 subjects: five to receive two 1000 mg doses of P218 48 hours apart and one to receive placebo. Subjects in subgroup 2 will not be treated until 24 hours after second IMP administration in the last subject of subgroup 1 and only upon decision of the PI after review of available safety data of subgroup 1.

Subjects will be admitted to the clinical unit in the morning of Day -1 and will be confined to the unit for 3 days thereafter (until Day 4, i.e. 36 hours after second IMP administration), for close safety monitoring and PK assessments. First administration of 1000 mg of P218 or placebo will take place on Day 1. Second administration of 1000 mg of P218 or placebo will take place 48 hours later, on Day 3. After discharge from the clinical unit on Day 4, subjects will be followed up with daily ambulatory visits to the clinical unit up to Day 9.

A schematic overview of the study design of Cohort 1 is shown in Figure 1. The assessments performed are summarized per visit in the Time and Events Schedule.

Figure 1: Schematic Overview of the Study Design for Cohort 1



Progression to Cohort 2 will be assessed by an SRT, who will review safety and tolerability after data up to Day 9 for Cohort 1 are available (see Section 5.8.1).

Cohorts 2 and 3

Cohort 2 will consist of three subgroups of subjects, to be enrolled sequentially: subgroup 1 will be composed of 2 subjects: one to receive two 1000 mg doses of P218 48 hours apart and one to receive placebo; subgroups 2 and 3 will be composed of 5 subjects each: four to receive two 1000 mg doses of P218 48 hours apart and one to receive placebo. Subjects in subgroup 2 will not be inoculated until 24 hours after second IMP administration in the last subject of subgroup 1. In subgroup 2 and subgroup 3, subjects will only be inoculated upon decision of the PI after review of available safety data of the previous subgroup, i.e. subgroup 1 and subgroup 2, respectively.

Cohort 3 will consist of two subgroups, each with 6 subjects, to be enrolled sequentially. Treatment will be allocated in a ratio of 3 active to 1 placebo. In each subgroup, at least 1 subject will receive placebo and the remaining subjects will receive two 100 mg doses of P218 48 hours apart. Subjects in subgroup 2 will not be inoculated until 24 hours after second IMP administration in the last subject of subgroup 1.

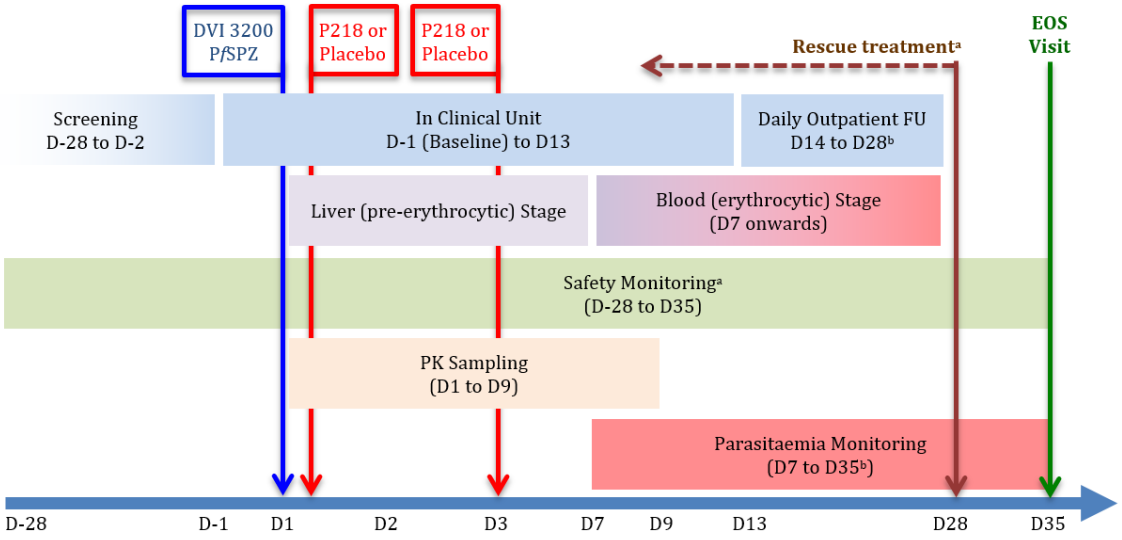
Subjects will be admitted to the clinical unit in the morning of Day -1 and will be confined to the unit until 12 days post the PfSPZ Challenge on Day 1 (i.e. until Day 13), for close safety monitoring and PK assessments. Each subject will be administered 3,200 *P. falciparum* sporozoites by DVI. First administration of P218 or placebo will take place 2 hours after PfSPZ Challenge inoculation. Second administration of P218 or placebo will take place 48 hours after first administration of P218 or placebo, on Day 3.

Safety, parasitaemia (see Section 2.2.1 for definition) and Malaria signs and symptoms (Malaria clinical score; see Attachment 1) will be assessed as of Day 7, i.e. during confinement in the unit until Day 13 and on daily ambulatory visits to the clinical unit as of Day 14, until development of positive parasitaemia or until Day 28 otherwise. If positive parasitaemia is confirmed, the subject will receive rescue therapy (see Section 5.2) and will continue to be monitored daily at the clinical unit until treatment success. Upon treatment success, daily visits to the clinical unit are stopped, except for subjects that first need to complete the 3-day rescue treatment regimen and associated assessments. All subjects will be assessed again for parasitaemia at the end-of-study (EOS) visit on Day 35. Subjects not developing positive parasitaemia until Day 28 will receive rescue therapy on that day. Instead of daily monitoring at the clinical unit until treatment success, these subjects will only be assessed again for parasitaemia at the EOS visit on Day 35. All subjects that receive rescue therapy will be asked non-leading questions to determine the occurrence of any AEs approximately two weeks after initiation of the rescue treatment, either during a planned study visit or by phone in case there is no planned study visit at that time.

Antimalarial rescue therapy (see Section 5.2) may be initiated whenever deemed necessary by the Investigators, e.g. if there is a concern regarding the safety of a study subject or if a study subject decides to withdraw from the study. Therapy may be amended according to the treating physician if the patient does not respond to treatment or the condition worsens.

A schematic overview of the study design of Cohorts 2 and 3 is shown in Figure 2. The assessments performed are summarized per visit in the Time and Events Schedule.

Figure 2: Schematic Overview of the Study Design for Cohorts 2 and 3



[a] All subjects that receive rescue therapy will be asked non leading questions to determine the occurrence of any AEs, approximately two weeks after initiation of the successful rescue treatment, either during a planned study visit or by phone in case there is no planned study visit at that time.

[b] Or until successful rescue treatment completion.

Progression to Cohort 3 will be assessed by the SRT, who will review safety, parasitaemia and Malaria signs and symptoms after data up to Day 35 for Cohort 2 are available, or after successful completion of the last rescue treatment, whichever is later (see Section 5.8.2).

3.2 DISCUSSION OF STUDY DESIGN

Dose Selection

Based on an *in vitro* experiment, P218 was expected to be active against *P. falciparum* infected hepatocytes from 48 hours onwards after incubation (Figure 3).

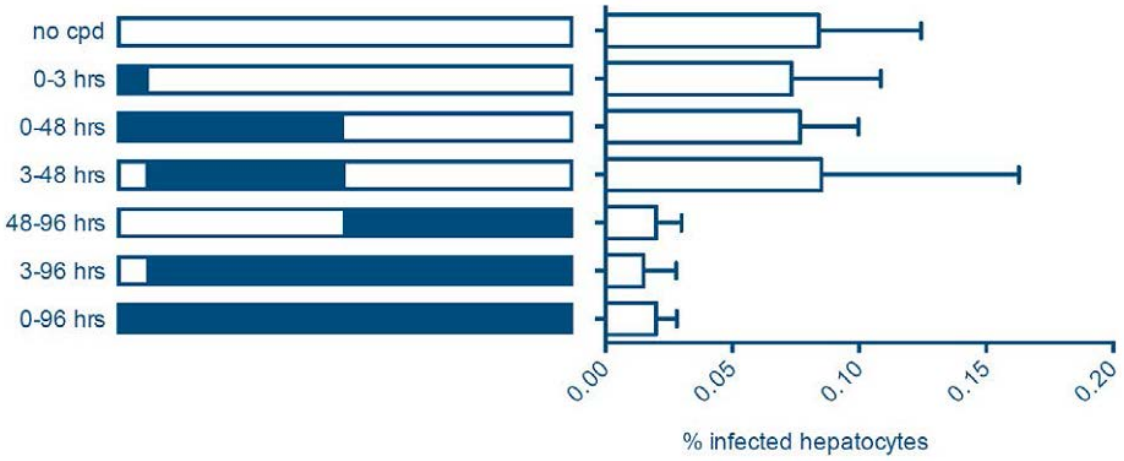


Figure 3: Incubation of *P. falciparum* infected hepatocytes with 100nM P218 at indicated time intervals (figure on the left), and % of remaining infected hepatocytes per time interval incubation (figure on the right).

In new *in vitro* experiments, the activity window of P218 was re-evaluated in *P. falciparum* (NF175) infected hepatocytes. The results depicted in Figure 4 suggest that P218 inhibits all stages of intra-hepatocytic development, from 3 hours onwards, post addition of sporozoites to the primary liver cells.

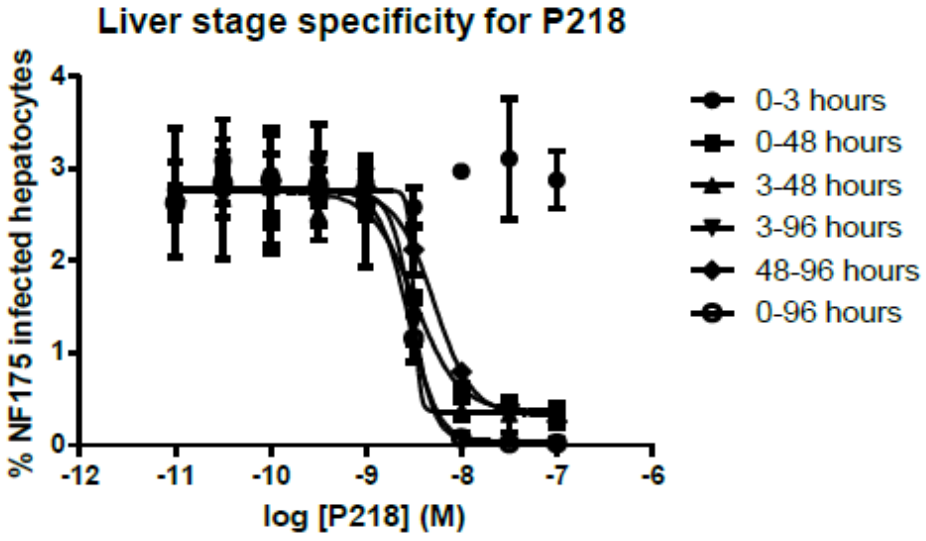


Figure 4: Activity window of P218 at different time intervals during infection and maturation of *P. falciparum* (NF175) infected hepatocytes.

In the completed FIH study with P218 conducted in the UK (MMV_P218_15_01/C16009), the highest dose tested was 1000 mg. The highest dose tested was considered safe and well tolerated and was associated with mean C_{max} and $AUC_{0\text{-}inf}$ values of 8.64 µg/ml and 17.8 µg·h/ml, respectively. That provided an exposure margin of 4.0-fold to the protocol defined stopping criteria of 71.9 µg·h/ml based on total cumulative $AUC_{0\text{-}336h}$ observed in the most sensitive species (dog). The single oral dose of 1000 mg of P218 was associated with a mean elimination half-life of 19.6 h, which is too short to allow for weekly or monthly oral dosing in a chemo-protection setting. Thus,

if proof of pharmacology is achieved in this human malaria challenge model, i.e. P218 activity against *P. falciparum* infection is clinically demonstrated, a long acting injectable depot formulation will be considered, allowing for slow-blood-release of P218 over at least one week and preferably, over one month following single injection [1].

Antimalarial efficacy against the blood stage *P. falciparum* has also been evaluated in a validated mouse model (Humanized SCID mouse) with established ED_{90} dose of 1.6 mg/kg/day and derived MPC and MIC of 4.4 ng/ml and 1.3 ng/ml, respectively. A human dose that could maintain blood concentrations above MIC was identified as a target minimal efficacious dose for chemo-protection against *P. falciparum* malaria. Using this approach and observed human PK in the completed FIH study, it was concluded that using the currently available oral formulation of P218, a human dose of 1000 mg would be needed to maintain in human subjects P218 plasma concentrations above the target mouse blood MIC. Based on P218 PopPK model simulations, two single doses of 1000 mg of P218 administered 48 hours apart would be needed in order to maintain P218 plasma concentrations above the target mouse blood MIC for a sufficient period of time during the *P. falciparum* liver stage to properly address the P218 chemoprotection against *P. falciparum* malaria proof of pharmacology (Figure 5) [14].

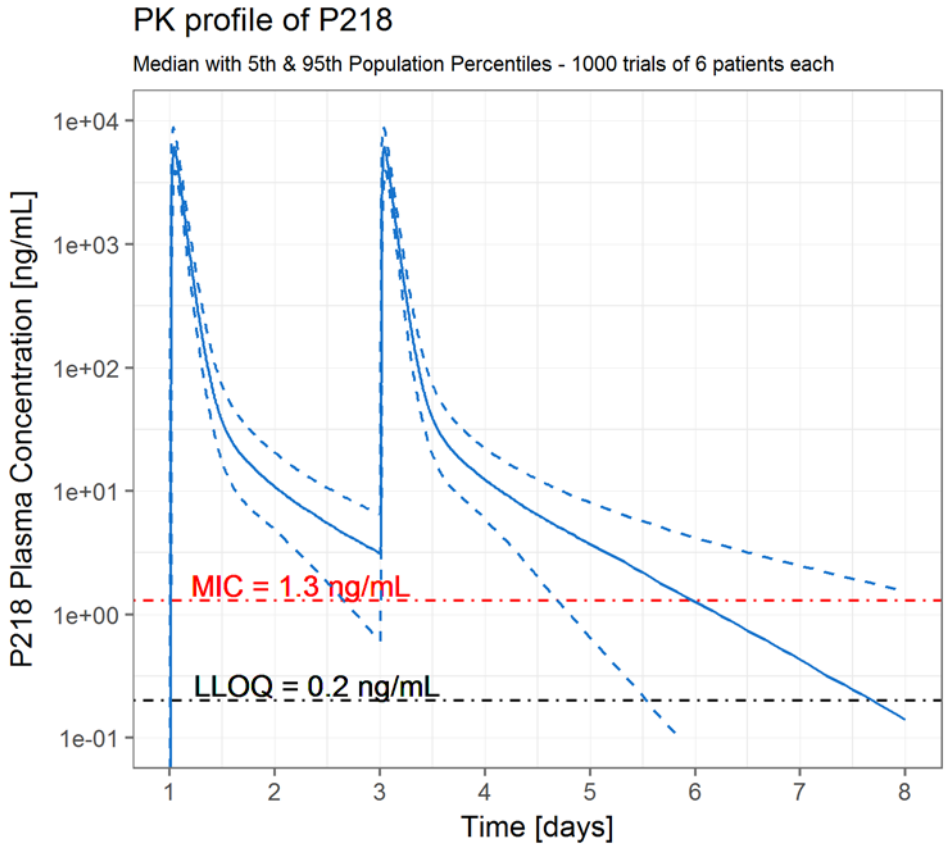


Figure 5: Simulations of the P218 plasma concentration over time following the oral administration of two single doses of 1000 mg of P218 when administered 48 hours apart on Day 1 and Day 3 using a Population PK model.

For the evaluation of the administration of two single doses of 1000 mg of P218 administered 48 hours apart, PopPK simulations predict that no accumulation of the

parent and major metabolites will occur and similar exposures to those associated with a 1000 mg single dose of P218 will be achieved (Table 1).

Table 1: Simulation of P218 and its Major Metabolites PK Parameters in Plasma After the Administration of Two Single Doses of 1000 mg of P218 Administered 48 Hours Apart Using a PopPK Model

Moiety	Parameter	Median	90% C.I.
P218	$AUC_{0\text{-}48h}$ (ug/L.h)	15708	[10997; 22495]
	$AUC_{48\text{-}96h}$ (ug/L.h)	15798	[11082; 22599]
	$AUC_{48\text{-}96h}/AUC_{0\text{-}48h}$	1.00	[1.00; 1.01]
	$AUC_{48h\text{-}inf}$ (ug/L.h)	15978	[11170; 22790]
P218 β acyl glucuronide	$AUC_{0\text{-}48h}$ (ug/L.h)	28101	[16680; 47373]
	$AUC_{48\text{-}96h}$ (ug/L.h)	28257	[16805; 47585]
	$AUC_{48\text{-}96h}/AUC_{0\text{-}48h}$	1.00	[1.00; 1.01]
	$AUC_{48h\text{-}inf}$ (ug/L.h)	28510	[16957; 48064]
	$AUC_{0\text{-}48h}/AUC_{0\text{-}48h,P218}$	1.80	[1.22; 2.60]
	$AUC_{inf}/AUC_{inf,P218}$	1.80	[1.22; 2.60]
P218-OH β acyl glucuronide	$AUC_{0\text{-}48h}$ (ug/L.h)	24061	[14118; 40130]
	$AUC_{48\text{-}96h}$ (ug/L.h)	24294	[14266; 40507]
	$AUC_{48\text{-}96h}/AUC_{0\text{-}48h}$	1.01	[1.00; 1.02]
	$AUC_{48h\text{-}inf}$ (ug/L.h)	24755	[14546; 41210]
	$AUC_{0\text{-}48h}/AUC_{0\text{-}48h,P218}$	1.54	[1.02; 2.24]
	$AUC_{inf}/AUC_{inf,P218}$	1.55	[1.03; 2.25]
P218-OH	$AUC_{0\text{-}48h}$ (ug/L.h)	1520	[937; 2490]
	$AUC_{48\text{-}96h}$ (ug/L.h)	1557	[961; 2540]
	$AUC_{48\text{-}96h}/AUC_{0\text{-}48h}$	1.02	[1.02; 1.03]
	$AUC_{48h\text{-}inf}$ (ug/L.h)	1611	[990; 2625]
	$AUC_{0\text{-}48h}/AUC_{0\text{-}48h,P218}$	0.10	[0.07; 0.14]
	$AUC_{inf}/AUC_{inf,P218}$	0.10	[0.07; 0.14]

C_{max} values are available in the PopPK report [14].
For P218 AUC cap: 71.9 µg•h/mL.

Therefore, the administration of two single doses of 1000 mg of P218 administered 48 hours apart was considered a safe dosing regimen of P218 to be evaluated in this clinical trial. However, since P218 oral single doses were assessed in the FIH study, the safety, tolerability and PK profile of 2 consecutive doses of 1000 mg of P218 administered 48 hours apart under fasted conditions will be characterized in Cohort 1. Then, for the second cohort of healthy men and women evaluating P218 as possible chemo-protective agent against *P. falciparum*, the most relevant dosing regimen with the currently available oral formulation was PfSPZ inoculation on Day 1 and administration of a 1000 mg oral dose of P218 under fasted conditions on Day 1 and Day 3.

Preliminary Cohort 2 data show that 3 subjects had breakthrough parasitaemia between Days 8 and 12, and 9 subjects had no positive parasitaemia, as assessed by PCR monitoring, up to Day 28. Although data are still blinded, these results suggest that there is no breakthrough in subjects treated with P218, if one assumes that the three subjects with positive parasitaemia received placebo, leading to conclude that P218 has chemoprotective activity. The new *in vitro* results are in good alignment with what was observed in Cohort 2.

Based on P218 PopPK model simulations, two single doses of 100 mg of P218 administered 48 hours apart on D1 and D3 would allow to better document the lowest efficacious exposure of P218. As shown in Figure 6, this regimen would maintain P218

plasma concentrations above the target mouse blood MIC for 24 hours after each P218 administration. This PK profile is expected to be associated with positive PCR in some subjects treated with active drug which will support a better estimation of the minimal inhibitory exposure of P218 required for liver activity.

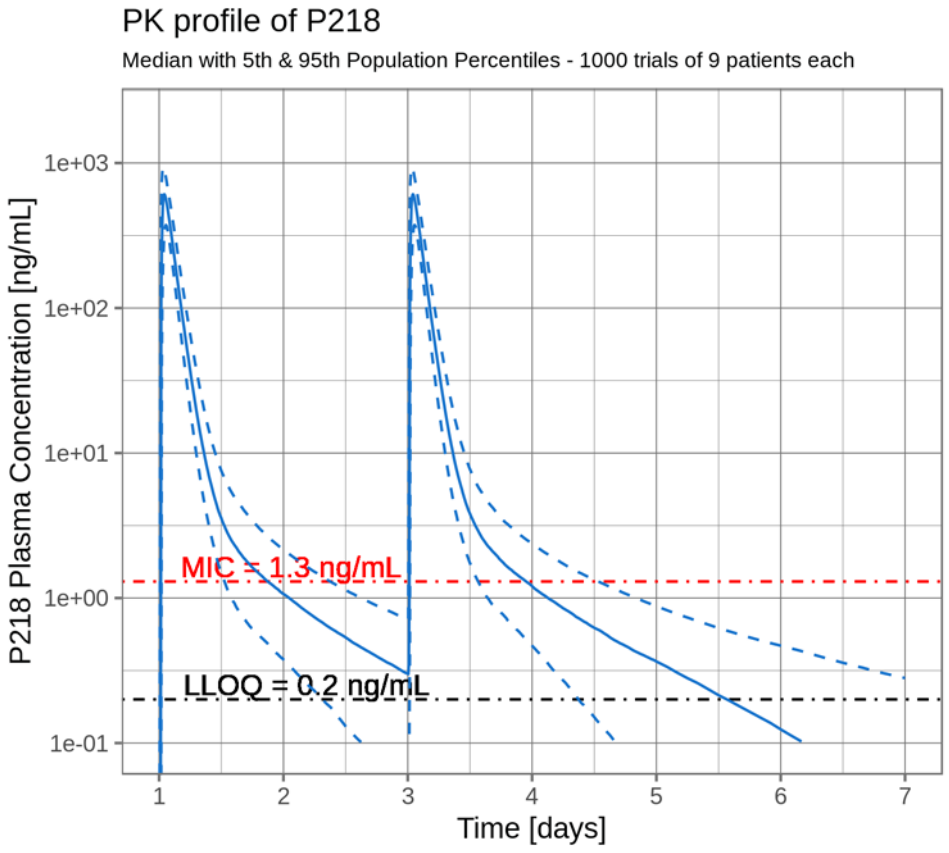


Figure 6: Population PK simulations of 1000 cohorts of 9 patients each with an administration of 2 single doses of 100 mg P218 at 100 mg administered 48 hours apart.

Table 2: Simulation of P218 and its Major Metabolites PK Parameters in Plasma After the Administration of Two Single Doses of 100 mg of P218 Administered 48 Hours Apart Using a PopPK Model

Moiety	Parameter	Median	90% C.I.
P218	$AUC_{0\text{-}48h}$ (ng/mL.h)	1556	[1085; 2240]
	$AUC_{48\text{-}96h}$ (ng/mL.h)	1565	[1090; 2251]
	$AUC_{48h\text{-}last}$ (ng/mL.h)	1578	[1098; 2270]
	$AUC_{48h\text{-}inf}$ (ng/mL.h)	1581	[1099; 2273]
	AUC_{inf} (ng/mL.h)	3135	[2187; 4516]
	C_{max} D1 (ng/mL)	742	[429; 1195]
	T_{max} D1 (h)	1	[0.5; 2.4]
	C_{max} D3 (ng/mL)	743	[430; 1195]
	T_{max} D3 (h)	49	[48.5; 50.4]
P218 β acyl glucuronide	$AUC_{0\text{-}48h}$ (ng/mL.h)	153	[93; 249]
	$AUC_{48\text{-}96h}$ (ng/mL.h)	157	[95; 255]
	$AUC_{48h\text{-}last}$ (ng/mL.h)	162	[98; 262]
	$AUC_{48h\text{-}inf}$ (ng/mL.h)	162	[99; 262]
	AUC_{inf} (ng/mL.h)	315	[192; 512]
	C_{max} D1 (ng/mL)	32	[19; 54]
	T_{max} D1 (h)	2.2	[1.8; 3.2]
	C_{max} D3 (ng/mL)	33	[19; 54]
	T_{max} D3 (h)	50.2	[49.8; 51.2]
P218-OH β acyl glucuronide	$AUC_{0\text{-}48h}$ (ng/mL.h)	2416	[1420; 4031]
	$AUC_{48\text{-}96h}$ (ng/mL.h)	2442	[1434; 4075]
	$AUC_{48h\text{-}last}$ (ng/mL.h)	2480	[1458; 4140]
	$AUC_{48h\text{-}inf}$ (ng/mL.h)	2484	[1459; 4145]
	AUC_{inf} (ng/mL.h)	4899	[2878; 8180]
	C_{max} D1 (ng/mL)	810	[434; 1465]
	T_{max} D1 (h)	1.3	[0.9; 2.6]
	C_{max} D3 (ng/mL)	811	[434; 1467]
	T_{max} D3 (h)	49.3	[48.9; 50.6]
P218-OH	$AUC_{0\text{-}48h}$ (ng/mL.h)	2797	[1618; 4710]
	$AUC_{48\text{-}96h}$ (ng/mL.h)	2813	[1631; 4736]
	$AUC_{48h\text{-}last}$ (ng/mL.h)	2836	[1648; 4778]
	$AUC_{48h\text{-}inf}$ (ng/mL.h)	2840	[1651; 4787]
	AUC_{inf} (ng/mL.h)	5637	[3269; 9484]
	C_{max} D1 (ng/mL)	1191	[648; 2064]
	T_{max} D1 (h)	1.2	[0.8; 2.5]
	C_{max} D3 (ng/mL)	1192	[649; 2064]
	T_{max} D3 (h)	49.2	[48.8; 50.5]

Therefore, for the third cohort of healthy men and women evaluating P218 as possible chemo-protective agent against *P. falciparum*, the most relevant dosing regimen with the currently available oral formulation was PfSPZ inoculation on Day 1 and administration of a 100 mg oral dose of P218 under fasted conditions on Day 1 and Day 3.

Placebo Control

Placebo control will be used to establish the frequency and magnitude of changes in clinical endpoints that may occur in the absence of active treatment. Note that this is also used to confirm "infectivity" of the PfSPZ inoculation, i.e. sort of internal validation of the inoculum (for a situation where all the subjects treated with P218 would be protected, one could question the "infectivity" of the inoculum).

Randomization and Blinding

Randomisation will be used to avoid bias in the assignment of subjects to treatment, to increase the likelihood that known and unknown subject attributes (e.g. demographic and baseline characteristics) are evenly balanced across cohorts and treatment groups, and to enhance the validity of statistical comparisons across cohorts and treatment groups.

Blinded treatment will be used to reduce potential bias during data collection and evaluation of clinical endpoints.

4. SELECTION OF STUDY POPULATION

Screening for eligible subjects will be performed within approximately 4 weeks prior to randomisation and up to Day -2.

Approximately 32 subjects are planned to be enrolled in one of 3 cohorts of 8, 12 and 12 subjects. A subject may be enrolled in one cohort only. Subjects will be randomised within each cohort in a 3:1 ratio to receive two consecutive administrations of either P218 or placebo.

For details on the sample size calculation, please refer to Section 9.2.

4.1 INCLUSION CRITERIA

Subjects meeting all of the following criteria are eligible to participate in this study:

1. Informed Consent Form signed voluntarily before any study-related procedure is performed, indicating that the subject understands the purpose of and procedures required for the study and is willing to participate in the study, including administration of rescue treatment.
2. Male or female, between 18 and 45 years old (extremes included) at screening.
3. Body weight of at least 50 kg and a body mass index (BMI) of 19 to 30 kg/m^2 (extremes included).
4. Good general health without clinically relevant medical illness, physical exam findings including vital signs, and laboratory abnormalities as determined by the investigator.
5. Willing to adhere to the prohibitions and restrictions (see Section 4.3) specified in this protocol, including willingness to stay confined to the inpatient unit for required duration and willingness to avoid to travel outside of Benelux during the study period.
6. Female subjects should fulfil one of the following criteria:
   a. At least 1 year post-menopausal (amenorrhea >12 months and follicle-stimulating hormone (FSH) >30 mIU/mL) prior to screening;
   b. Surgically sterile (bilateral oophorectomy, hysterectomy or tubal ligation);
   c. Will use contraceptives as outlined in inclusion criteria 7 and 8.
7. Female subjects of childbearing potential must agree to the use of a highly effective method of birth control from screening visit to until 40 days after the last dose of IMP (covering a full menstrual cycle of 30 days starting after 5 half-lives of last dose of IMP).

   Note: Highly effective birth control methods include: combined (estrogen and progestogen containing) oral/intravaginal/transdermal 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.
8. Male subjects who are sexually active with a female partner of childbearing potential must agree to the use of an effective method of birth control from the day of the first IMP dose until 100 days thereafter (covering a full sperm cycle of 90 days starting after 5 half-lives of last dose of IMP).

   Note: Medically acceptable methods of contraception that may be used by the subject and/or partner include sterilization and vasectomy or a double barrier option combining oral

contraceptive, contraceptive vaginal ring, contraceptive injection, intrauterine device or etonogestrel implant.

9. Female subject has a negative pregnancy test at screening and upon admission in the clinical unit.

   Note: Pregnancy testing will consist of a serum β-human chorionic gonadotropin (β-HCG) test at screening and urine β-HCG tests at other visits, in all women.

Inclusion Criteria - CHMI specific:

10. Different ways of being reachable 24/7 (e.g. by mobile phone, regular phone or electronic mail) during the whole study period.

4.2 EXCLUSION CRITERIA

Subjects meeting any of the following criteria are excluded from participation in this study:

1. Nursing (lactating) women.
2. Participation in any other clinical drug or vaccine study within 30 days (or five half-lives for drugs) preceding the first dose of IMP (whichever is longer), or plans to participate in other investigational drug or vaccine research during the study period.
3. Blood product donation to any blood bank during the 8 weeks (whole blood) or 4 weeks (plasma and platelets) prior to admission in the clinical unit.
4. ECG outside normal range and deemed clinically relevant by the investigator. Examples of clinically significant ECG abnormalities for this study include:
   a. PR-interval >220 ms;
   b. QRS-complex >120 ms;
   c. QT interval corrected according to Bazett's formula (QTcB) or QT interval corrected according to Fridericia's formula [3] (QTcF) >450 ms;
   d. Pathologic Q wave;
   e. Significant ST-T wave changes;
   f. Left or right ventricular hypertrophy;
   g. Non-sinus rhythm except isolated premature atrial contractions and ventricular extrasystole <2 per 10 s ECG lead;
   h. Incomplete left bundle branch block, or complete or intermittent right or left bundle branch block;
   i. Second or third degree A-V heart block.
5. Seropositive human immunodeficiency virus (HIV) (antibody and antigen), hepatitis B virus (HBV) (hepatitis B surface antigen [HBsAg]) or hepatitis C virus (HCV) (antibody) tests.
6. History or presence of diagnosed food or known drug allergies (including but not limited to allergy to any of the antimalarial rescue medications to be used in the study, see Section 5.2), or history of anaphylaxis or other severe allergic reactions.

   Note: Subjects with seasonal allergies/hay fever, house dust mite or allergy to animals that are untreated and asymptomatic at the time of dosing can be enrolled in the study.

7. History of convulsion or severe head trauma.

   Note: A medical history of a single febrile convulsion during childhood is not an exclusion criteria.

8. 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 (Attachment 2) will be used as an objective tool for the assessment of depression at screening. In addition to the conditions listed above, subjects 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. Subjects 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.

9. A medical, occupational or family problem as a result of alcohol or illicit drug abuse during the past 12 months or current alcohol or illicit drug abuse or addiction (positive alcohol breath test or positive drug screen for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine or opiates at screening or upon check-in at the clinical unit).

   Note: Excessive use of alcohol is an intake of >21 units per week for males and >14 units per week for females where one alcohol unit is defined as 10 mL or 8 g of pure alcohol. A single unit is equal to one 25-mL (single) measure of whisky (alcohol by volume [ABV] 40%), or a third of a pint of beer (190 mL; ABV 5-6%) or half a standard (175 mL) glass of wine (ABV 12%).

10. Subjects are non-smokers or ex-smokers for more than 90 days prior to screening or smoke no more than 5 cigarettes per day. If users of nicotine products (i.e. spray, patch, e-cigarette, etc.) they should use the equivalent of no more than 5 cigarettes per day. Subjects must agree to abstain from smoking while in the unit.

11. Use of any prescription drugs, herbal supplements (e.g. St John's Wort) or over-the-counter medication within 7 days or five half-lives (whichever is longer) prior to the first IMP administration, or an anticipated requirement for the use of these during the course of the study (See Section 6.2).

    Note: If necessary, the incidental use of non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol (2g/day, 10 gr/week), vitamins and topical treatments may be acceptable after approval by the study Sponsor and will be documented in the eSource system. The use of nutritional supplements during this time that are not believed to have the potential to affect subject safety nor 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.

12. Any surgical or medical condition possibly affecting drug absorption (e.g. cholecystectomy, gastrectomy, bowel disease), distribution, metabolism or excretion.

13. Any history of gallbladder disease, including cholecystitis and/or cholelithiasis.

14. History of megaloblastic anaemia or folate deficiency.

15. Personnel (e.g. investigator, sub-investigator, research assistant, pharmacist, study coordinator or anyone mentioned in the delegation log) directly involved in the conduct of the study.
16. Any condition that in the opinion of the investigator would jeopardize the safety or rights of a person participating in the trial or would render the person unable to comply with the protocol.

Exclusion Criteria - CHMI specific:

17. Glucose-6-phosphate dehydrogenase (G6PD) deficiency (due to possible hemolysis induced by primaquine treatment at study end in G6PD deficient subjects).
18. Personal history of malaria.
19. Volunteer has travelled to or lived in a malaria-endemic area for more than 4 weeks during the 12 months prior to first IMP administration, or spent any time in an endemic area during the 4 weeks prior to first IMP administration.
20. Plans to travel to malaria-endemic region during the study period up to last follow-up visit.
21. Previous participation in any malaria vaccine or CHMI study.
22. Falling in moderate or higher risk category for a fatal or non-fatal cardiovascular event within 10 years (≥5%) determined by a validated risk estimation system e.g. SCORE [13].
23. Use of medications known to interact with atovaquone-proguanil (Malarone®), artemether-lumefatrine (Riamet®) or primaquine (Primaquine®) such as cimetidine, metoclopramide or antacids, or an anticipated requirement for the use of these at any point during the study period (see Section 6.2).
24. Use of systemic antibiotics with known antimalarial activity within 30 days (or 5 half-lives whichever is longer) of first IMP administration (e.g. trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones or azithromycin) or an anticipated requirement for the use of these during the study period (see Section 6.2).
25. Receipt of blood or blood-derived products (including immunoglobulin) within 3 months prior to screening. Receipt of packed red blood cells given for an emergent indication in an otherwise healthy person, and not required as ongoing treatment is not exclusionary (for example packed red blood cells emergently given during an elective surgery).

Note: In case of an out-of-range clinical laboratory test, vital sign or ECG value that will determine a subject's eligibility, or in case of a positive drug screen, a retest or expert evaluation can be requested. Results of any retest must be available prior to inoculation. The result of the retest will be considered for subject eligibility at the investigator's discretion. Subjects can be rescreened at the discretion of the investigator.

4.3 ADDITIONAL CONSTRAINTS

Subjects will have to comply with the following restrictions:

Meals and Dietary

1. Safety clinical laboratory blood tests will be performed after fasting for at least 8 hours.
2. IMP will be administered after fasting for at least 8 hours. Water is restricted from 1 hour predose and is allowed ad libitum as of 2 hours post dose. Snacks are allowed from 2 hours post dose and a meal can be taken 4 hours after dosing.
3. During confinement in the clinical unit, no food intake in addition to the standard meals and snacks provided by the study personnel will be allowed.
4. Subjects will abstain from xanthine-containing food or beverages (e.g. coffee, tea, cola, chocolate) from 24 hours prior to screening and 24 hours prior to the first IMP administration until collection of the last PK sample.
5. Subjects will abstain from energy drinks containing taurine or glucuronolactone from 24 hours prior to screening and from 24 hours prior to the first IMP administration until collection of the last PK sample.
6. Subjects will abstain from drinking alcohol from 24 hours prior to screening and 24 hours prior to the first IMP administration until collection of the last PK sample.
7. Subjects will abstain from eating or drinking grapefruit or grapefruit-related citrus fruits (e.g. Seville oranges, pomelos) from 30 days prior to the first IMP administration and for the whole duration of the study.
8. Ban of transplantation until successful rescue.

Activity

9. Subjects will be confined from Day -1 in the morning to Day 4 (i.e. 36 hours after second IMP administration) in Cohort 1 and from Day -1 in the morning to 12 days post the PfSPZ Challenge on Day 1 in Cohorts 2 and 3, unless they are withdrawn from the study.
10. Authorization to leave the clinical unit will be given by the Investigator.
11. Subjects will abstain from smoking a tobacco product or marijuana from 24 hours prior to the first IMP administration until end of confinement.
12. Subjects will abstain from any strenuous activity (e.g. weight lifting, aerobics, football, endurance training sessions) or unaccustomed physical exercise from 48 hours prior to screening and from 48 hours prior to the first IMP administration until collection of the last PK sample.

Other

13. Information on prohibited therapies can be found in Section 6.2.

5. TREATMENT(S)

Manufacturing, packaging and labelling of P218/placebo (IMP) and PfSPZ Challenge incoculum will be done under the responsibility of the Sponsor and the biotechnology company Sanaria (USA) [6,7], respectively.

5.1 PHYSICAL DESCRIPTION OF THE IMP

5.1.1 *P218/placebo*

P218 and placebo capsules are prepared in accordance with Good Manufacturing Practice (GMP) and supplied by the Sponsor as bulk to the pharmacy of the clinical unit, including batch certificates, as:

- P218, 50 mg and 250 mg capsules for oral administration;
- Placebo to match P218 capsules for oral administration.

Blinded treatment regimens for each subject will be prepared at the pharmacy of the clinical unit. Please refer to the Dispensing Protocol for further details.

Due to the favourable physicochemical properties of P218, the active pharmaceutical ingredient (API) was formulated in capsules without excipients. Capsules (size 0 HPMC capsules comprising a VCaps®Plus Swedish orange body cap) were produced via hand-filling of API and contained 50 mg or 250 mg of P218.

P218 capsules (50 mg and 250 mg) are packaged into high density polypropylene containers with a twist-off polypropylene screw cap lid with up to 30 units per container. A detailed description of the physical, chemical and pharmaceutical properties of P218 can be found in Section 4 of the IB [6] and in the Investigational Medicinal Product Dossier (IMPD) [8].

Placebo capsules will be used for the purpose of the clinical trial. To preserve blinding, placebo capsules will be matched to the drug product with regard to appearance and taste. They are contained in the same packaging as the drug product.

5.1.2 *PfSPZ Challenge*

The PfSPZ Challenge consists of aseptic, cryopreserved *P. falciparum* sporozoites used for CHMI trials and is produced by the biotechnology company, Sanaria (USA) [7].

In brief, the manufacturing process includes the production, under traditional environmental condition, of eggs from a colony of *A. stephensi* mosquitos housed in a controlled environmental chamber. Surface disinfection of the eggs is performed by exposure to chemical agents in a Class II biosafety cabinet (BSC). Thereafter, all materials and product are handled using aseptic methods to ensure that contaminating microorganisms are not introduced to, and carried through, the process. Surface-disinfected eggs are inoculated into sterile, vented flasks containing aseptic growth medium. The eggs hatch and develop into pupae, which are transferred to an adult mosquito container from which the adult mosquitoes emerge.

These adult mosquitos, which have been raised under aseptic conditions, are fed *P. falciparum* gametocyte-infected blood in a BSC in a high-security insectary in Rockville, Maryland, USA. The *P. falciparum* gametocyte-infected blood is produced

from cultures of the *P. falciparum* strain NF54, derived from a master cell bank of the well characterised *P. falciparum* strain NF 54. Infected adult mosquitos are maintained under aseptic conditions until *P. falciparum* sporozoites migrate to the salivary glands. The salivary glands from the *P. falciparum* sporozoite infected mosquitos are removed by hand dissection and then triturated to release the *P. falciparum* sporozoites.

The sporozoites are purified, counted, and at a specified concentration, cryopreserved. Cryopreservation commences with the addition of cryoprotective additives to the purified sporozoites to produce the PfSPZ Challenge product.

The diluent for PfSPZ Challenge is composed of phosphate buffered saline (PBS) and human serum albumin (HSA). Vials of PBS and HSA will be shipped to the clinical unit, where diluent composed of PBS and HSA is prepared according to a local Standard Operational Procedure, as described in the Laboratory Manual of Sanaria (Storage, Preparation and Administration of PfSPZ Challenge).

PBS that is manufactured in compliance with GMP and according to upstream processing specifications is purchased by Sanaria. Every lot of PBS is supplied with a batch certification that is reviewed and approved upon receipt of Sanaria. The PBS is stored at ambient temperature in a controlled room.

HSA (25%), approved for parenteral, intravenous administration to humans, is purchased by Sanaria. Every lot of HSA is supplied with a Certificate of Analysis that is reviewed and approved upon receipt of Sanaria. HSA vials are stored at ambient temperature in a controlled room.

The PfSPZ Challenge is stored in liquid nitrogen vapour phase at -140°C to -196°C until it is shipped to the clinical study unit. Shipment is in compliance with U.S. Food and Drug Administration (FDA), U.S. Department of Transportation and United Nations transport guidelines for shipping bio-hazardous materials on dry ice and liquid or vapour phase nitrogen.

5.2 ANTIMALARIAL RESCUE MEDICATION

The clinical unit will be responsible for acquiring the anti-malarial drugs, Riamet®, Malarone® and Primaquine®:

- Subject will be prescribed with Riamet® (20 mg artemeter and 120 mg lumefantrine) to ensure parasite clearance prior to the end-of-study evaluation. Tablets for oral use will be taken over 3 days. The first dose should be taken as soon as possible and should be followed by five further doses approximately 8, 24, 36, 48 and 60 hours after the first dose. Drug administration should be immediately followed by a meal or drinks rich in fat (e.g. milk).
- If an intolerance or contraindication to Riamet® develops, Malarone® (250 mg atovaquone and 100 mg proguanil hydrochloride; tablets for oral use) will be administered for 3 consecutive days.
- To ensure complete clearance of gametocytes (a low probability event in this study due to early treatment at low parasitemia levels), subjects will receive a single dose of Primaquine® (26.4 mg primaquine phosphate equivalent to 15 mg primaquine base; tablets for oral use, registered product in Canada) on the first day of rescue treatment or on Day 28 together with the rescue treatment, for subjects who do not develop positive parasitaemia until this day.

The PfSPZ Challenge product consists of a strain *P. falciparum* sporozoites used for CHMI trials that is known to be sensitive to the rescue treatment described above [7].

Rescue treatment will be administered and monitored on the occasion of daily ambulatory visits to the clinical unit. The required anti-malarial drug intakes 36 and 60 hours after the first dose, can be done at home. Subjects will be notified by a study nurse via phone/text message/e-mail when it is time for dosing and must confirm study drug intake. On commencement of antimalarial rescue medication, and in addition to the assessments described in Section 7 and the Time and Events Schedule, subjects will report to the clinical unit to enable the following assessments to be performed, where X^0 is the time prior to the first dose of treatment, X^{24} is after 1 day of treatment, X^{48} is after 2 days of treatment and X^{72} is after completion of the rescue treatment:

- Blood sampling for the following parameters (only for subjects who develop asexual parasitaemia):
  - Malaria parasitaemia by qPCR (X^0, X^{24}, X^{48} and X^{72} and EOS visit);
  - Malaria parasitaemia by TBS (X^0, X^{24}, X^{48} and X^{72} and EOS visit);
  - Troponin I (X^{48} and X^{72}) (see Section 7.4.2)
- Questioning regarding signs and symptoms of malaria and calculation of a malaria clinical symptom score (at all time points for subject who were treated for positive parasitaemia only).
- Recording of adverse events and concomitant medications (all time points).

TBS microscopy and qPCR assessments of parasitaemia will be carried out daily until treatment success (defined as one negative qPCR outcome for asexual parasites, i.e. 0 parasites per mL, confirmed by TBS microscopy). Upon treatment success, daily visits to the clinical unit are stopped, except for subjects that first need to complete the 3-day rescue treatment regimen and associated assessments. All subjects will be assessed again for parasitaemia at the EOS visit on Day 35.

In subjects who do not develop positive parasitaemia until Day 28 of the study, rescue treatment will be initiated on Day 28. Instead of daily qPCR, TBS and Troponin I assessments until treatment success, for these subjects only a final qPCR will be performed at the EOS visit on Day 35. Antimalarial rescue medication intakes can be done at home. Subjects will be notified by a study nurse via phone/text message/e-mail when it is time for dosing and must confirm study drug intake.

All subjects that receive rescue therapy will be asked non leading questions to determine the occurrence of any AEs approximately two weeks after initiation of the rescue treatment, either during a planned study visit or by phone in case there is no planned study visit at that time.

All subjects must consent to rescue treatment. Even in case of withdrawal from the study, all challenged subjects are to receive rescue treatment as soon as possible, including all appropriate visits and assessments, and phone call as required (see above).

5.3 PACKAGING AND LABELING

5.3.1 *P218/placebo*

The study drugs will be repackaged into individual subject doses according to the dose level and randomisation schedule by the pharmacy staff at the clinical unit.

The labelling of the IMPs will be in compliance with GMP specifications, as described in The Rules Governing Medicinal Products in the European Union, Volume 4, Annex 13, Investigational Medicinal Products, and any other or local applicable regulations.

Sample label(s) will be submitted to the Belgian health authorities according to the submission requirements.

5.3.2 *PfSPZ Challenge*

The PfSPZ Challenge product is dispensed into screw-cap vials containing 15,000 or 50,000 sporozoites in a 20 µL aliquot. PfSPZ Challenge is stored in liquid nitrogen vapour phase at -140°C to -196°C.

Transfer of PfSPZ Challenge from its storage site to the clinical unit will follow local Standard Operational Procedure, as described in the Laboratory Manual of Sanaria.

At the clinical unit, the liquid nitrogen vapour phase container will be monitored. Receipt of the PfSPZ Challenge will be documented on a tracking log by study staff.

5.4 STORAGE AND DRUG ACCOUNTABILITY

5.4.1 *P218/placebo*

The Investigator (or his/her designee) is responsible for the safe storage of all study drugs assigned to the clinical unit, in a locked, secure storage facility with access limited to those individuals authorized to dispense the study drugs, and maintained within the appropriate ranges of temperature. All study drugs must be stored as specified at delivery and in the original packaging.

Study drugs must be stored at ambient temperature (15-30°C or 59-86°F), away from light.

Regular temperature logging of the study drug storage room at the clinical unit should be performed. In case a deviation in storage conditions should occur, the clinical unit must not further dispense the affected study drug and notify the Sponsor.

The Investigator is responsible for ensuring that all study drugs received at the clinical unit are inventoried and accounted for throughout the study.

Study drugs should be dispensed under the supervision of the Investigator, a qualified member of the clinical staff, or by a hospital/clinic pharmacist. The Investigator must maintain accurate records demonstrating date and amount of drugs supplied to whom and by who. Study drugs will be supplied only to subjects participating in the study.

The Sponsor's designated site monitor will periodically check the supplies of study drugs held by the Investigator or pharmacist to ensure accountability and appropriate storage conditions of all study drugs used.

Unused study drugs must be available for verification by the site monitor during on-site monitoring visits.

After the last visit of the last subject in the study (LPLV), any unused study drug will be returned to the Sponsor, or destroyed at the clinical unit with the Sponsor's written permission (in this case a certificate of destruction will be provided and filed in the Trial Master File [TMF]).

5.4.2 *PfSPZ Challenge Agent*

PfSPZ Challenge is stored in liquid nitrogen vapour phase at -140°C to -196°C. See also Section 5.3.2.

5.5 RANDOMIZATION AND BLINDING

At screening, subjects receive a unique screening number using the letter S and a number ranging from 001 to 999. Subjects who are rescreened will be assigned a new screening number.

Subjects will be assigned to 1 of 3 cohorts. Within each cohort, they will be randomised in a 3:1 ratio to receive two consecutive administrations of either P218 or placebo. Allocation of each subject to a given treatment will be described in a randomisation list prepared prior to study start by SGS Life Sciences Secure Data Office using SAS® software (SAS Institute Inc., Cary, NC, USA).

The randomisation will be balanced using randomly permuted blocks across the treatment groups.

Based on this randomisation code, the study drug will be packaged and labeled. Medication code numbers will be preprinted on the study drug labels and assigned as subjects qualify for the study and are randomly assigned to treatment.

In agreement with the Sponsor, additional subjects may be recruited in each cohort, to replace discontinuations for non-safety reasons and achieve cohort sizes of 12. Replacement subjects will receive the number of the subject to be replaced, increased by 100, and will be administered the same treatment.

Blinding will be achieved with placebo identical in appearance.

The randomisation list will be retained by SGS Life Sciences Secure Data Office until the end of the study (database lock). One copy of the randomisation list will be sent in a sealed envelope to the site pharmacist before the start of the study. Another copy will also be sent before the start of the study in a sealed envelope to the bioanalytical laboratory responsible for plasma drug determination.

An individual disclosure envelope will be supplied to the Investigator for each subject (or randomisation number) and will contain the medication assignment for that subject. The Investigator will keep the treatment code envelopes in a locked, secure storage facility. A treatment code envelope can only be opened in an emergency situation where the Investigator considers it essential to know what treatment the subject was receiving. The medical monitor shall be notified promptly if a treatment code envelope is opened. It is recommended that the Investigator contacts the Sponsor before opening an envelope, if possible. The Investigator must document the date, time, and reason for the unblinding in the eSource system. If the code is broken by the Investigator or by someone of his/her clinical staff, the subject must be withdrawn from the study and must be followed as appropriate. If the code is broken by the Sponsor for safety reporting purposes, the subject may remain in the study.

Upon completion of the study, the monitor needs to check the completeness and status of the envelopes and once this is done the monitor can retrieve the envelopes and have them destroyed.

5.6 DOSE AND ADMINISTRATION

5.6.1 *P218/placebo*

A rationale for the dose of study drug selected in this study is provided in Section 3.2.

On each scheduled dosing (see Time and Events Schedule), subjects will take simultaneously 4 capsules (either placebo or a total of 1000 mg P218) p.o. in Cohorts 1 and 2 or 2 capsules (either placebo or a total of 100 mg P218) p.o. in Cohort 3 after at least 8-hour fasting. After administration, a minimum of 2 hours of fasting will be required.

Subjects will be asked to take the study drugs as indicated in the Time and Events Schedule under the supervision of the clinical staff. Study drug administration will be done with 240 mL of non-carbonated water and in a fasted state (overnight fast for at least 8 hours; intake of water will be allowed until 1 hour before the administration of the study drugs). After intake of the study drugs, the subjects will be asked to maintain an upright position for at least 15 minutes. Thereafter, they will be asked to remain seated in bed for 2 hours.

Fasting conditions will be maintained for 2 hours, including drinking water that will be allowed from 2 hour post dose onwards (ad libitum but approximately 1500mL/24 hours).

For Cohort 1, a standardized snack, lunch, snack, dinner and snack will be served at approximately 2, 4, 8, 10 and 12 hours, respectively, after the administration of study drugs. For Cohorts 2 and 3, a standardized snack, lunch, dinner and snack will be served at approximately 2, 4, 8 and 10 hours, respectively, after the administration of study drugs.

Any deviation from the treatment regimen defined in the protocol must be documented in the eSource system.

5.6.2 *PfSPZ Challenge*

Immediately prior to use, PfSPZ Challenge in cryovials (each of which contains 15,000 or 50,000 sporozoites), will be thawed individually by partial submersion of the vials for 30 seconds in a 37°C ± 1°C water bath. Designated, trained study staff will then prepare, dilute (if necessary) and dispense the PfSPZ Challenge to clinical staff at the clinical unit according to local standard Operational Procedures and as described in the Laboratory Manual of Sanaria.

The PfSPZ Challenge (3,200 *P. falciparum* sporozoites per subject) will be administered intravenously by DVI. The study staff administering the PfSPZ Challenge will wear gloves and eye protection. Advanced life support drugs and resuscitation equipment will be immediately available in the event of any subjects experiencing an anaphylactic reaction to the challenge.

5.7 TREATMENT COMPLIANCE

To ensure treatment compliance, all study drug intakes (P218/placebo and PfSPZ Challenge) will be supervised by the Investigator or his/her designee.

5.8 SAFETY REVIEW TEAM

The SRT will review safety data during the study. The required data will be detailed in a separate study specific Safety Management Plan and will be sent to the SRT at least 48 hours prior to an SRT meeting.

Initially the data will be reviewed blinded, but if it is considered necessary by the SRT due to a safety concern, either individual subjects or the entire cohort may be unblinded to the SRT to enable their decision-making. Before breaking the code, the potential decisions and actions should be determined and documented.

The decision of the SRT to progress or not to the next cohort will be taken in consensus between the members of the SRC.

5.8.1 *For Progression From Cohort 1 to Cohort 2*

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

The SRT will meet after data up to Day 9 for Cohort 1 are available to review safety and tolerability in Cohort 1 and determine whether progression to Cohort 2 is indicated.

Specific guidelines for toxicity rules that need to be taken into account in taking the decision to proceed or not with Cohort 2 are provided in Section 5.8.1.2. If no concerns are identified during SRT review, progression to Cohort 2 will take place.

5.8.1.1 *DATA REQUIREMENTS FOR PROGRESSION FROM COHORT 1 TO COHORT 2*

A minimum of 168 hours post dose safety data from a minimum of 6 subjects (of which a minimum of 4 will have received the active compound) from Cohort 1 will be required for progression of the study to Cohort 2.

Assessments of safety will be determined from AEs, vital sign parameters (temperature, blood pressure and heart rate), 12-lead ECGs and clinical laboratory parameters (hematology, coagulation, biochemistry and urinalysis). Assessments will also be determined from other relevant clinical tests conducted on a case-by-case basis or other exploratory samples, additional data that may be available at the time of review.

5.8.1.2 *TOXICITY RULES*

For the purpose of this protocol, toxicity means clinically significant and at least possibly drug-related AE(s). An overview of rules to guide progression to Cohort 2 based on observed toxicities in Cohort 1 is provided in Table 3.

Table 3: Group Toxicity Rules for Progression From Cohort 1 to Cohort 2.

			Number of subjects		Toxicity Rules for progression to Cohort 2	
Grade	**Severity**	**Seriousness**	**Number of subjects affected in one SOC**	**Total Number of subjects affected**	**Showing signs of reversibility within 7 days**	**Action**
1	Mild	N/A	Any	Any	N/A	No action required
2	Moderate	N/A	2	≤3	Yes	Progression to Cohort 2 on hold until results of full dosing regimen of Cohort 1 are available, to which toxicity rules will be applied. Progression can then proceed, unless the data meet suspension rules in which case a substantial amendment is required.
			≥3	≥4	Yes	Progression to Cohort 2 requires substantial amendment.
			N/A	1	No	Progression to Cohort 2 on hold until results of full dosing regimen of Cohort 1 are available, to which toxicity rules will be applied. Progression can then proceed, unless the data meet suspension rules in which case a substantial amendment is required.
			N/A	≥2	No	Progression to Cohort 2 requires substantial amendment.
3	Severe	Not serious	N/A	1	Yes	Progression to Cohort 2 on hold until results of full dosing regimen of Cohort 1 are available, to which toxicity rules will be applied. Progression can then proceed, unless the data meet suspension rules in which case a substantial amendment is required.
			N/A N/A	≥2 ≥1	Yes No	Progression to Cohort 2 requires substantial amendment.
3	Severe	Serious	N/A	≥1	N/A	
4 or 5	Life-threatening or death -related	Serious	N/A	≥1	N/A	Study suspended.

Local laboratory normal values will be applied. Abnormal laboratory and other tests and measurements will be repeated whenever feasible and or appropriate, prior to grading in order to ensure consistency and to exclude technical errors. Diurnal variations in laboratory variables and other measurements as well as baseline status and conditions will be taken into account when assessing whether abnormalities constitute a drug related AE and when grading, if applicable.

5.8.2 *For Progression From Cohort 2 to Cohort 3*

For Cohort 2, the SRT members will include at least the PI, the MMV Medical Director 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, a pharmacokineticist and/or a statistician may be consulted by the SRT as necessary.

The SRT will review safety, parasitaemia and malaria signs and symptoms data during the study. The SRT will meet after the completion of all Day 35 assessments in Cohort 2 or after successful completion of the last rescue treatment, whichever is later, to review safety, parasitaemia and malaria signs and symptoms data in Cohort 2 and determine whether progression to Cohort 3 is indicated. If no safety concerns are identified during SRT review, progression to Cohort 3 will take place. Initiation of Cohort 3 will be put on hold and further review will be conducted by the SRT, if any of the following are observed in Cohort 2:

- PfSPZ Challenge-related or IMP-related SAE, or
- Any other critical PfSPZ Challenge-related finding that may place subjects at risk in subsequent subgroups (in the next cohort or within the same cohort), or
- Two or more IMP-related severe (Grade 3 or higher), same organ class adverse events.

If after data review the SRT confirms that any of the above has been met and relationship with the PfSPZ and/or the IMP is definitively established, progression to Cohort 3 can be either stopped or authorized at a lower dosage regimen (de-escalation possible based on safety observations).

5.8.3 *Final SRT Review at Study End*

The SRT will also meet at the end of the study when data from Cohort 3 are available for a final assessment in order to review the study results and share feedback on possible safety signals/events.

Throughout the study, the SRT can be convened at the request of any member should they have cause for concern regarding subject safety in relation to the challenge agent, where no other cause can be attributed. In the event that it is not possible to quickly convene the SRT for a review of the data, or not all the data are available, the study will be interrupted or temporarily halted, at the discretion of the PI or Sponsor, at any time.

6. PRIOR AND CONCOMITANT THERAPY

All therapies (prescriptions and over-the-counter medications, including herbal preparations/treatments) other than the IMP and antimalarial rescue medication, administered from informed consent until the last study visit must be recorded in the source documents and in the concomitant therapy section of the eSource system (name of the drug, dosage, route and dates of administration).

Medications taken during the 28 days prior to first IMP administration will be recorded in the eSource system as previous medications. Medications taken after this time will be recorded as concomitant medications.

Female subjects of childbearing potential and non-vasectomized male subjects having a female partner of childbearing potential must agree to the use of an effective method of contraception throughout the study, as outlined in Section 4.1. The use of oral, injectable and implantable hormonal contraceptives is to be recorded in the eSource system.

6.1 PERMITTED CONCOMITANT THERAPIES

Subjects will abstain from using any medications (prescription or over-the-counter) or herbal remedies 7 days prior to first administration of the IMP as described in the exclusion criteria of this protocol (see Section 4.2). If necessary, the incidental use of NSAIDs, paracetamol (2g/day, 10 gr/week), vitamins, topical treatments, and the use of nutritional supplements may be acceptable after approval by the study Sponsor.

Except for the IMP, antimalarial rescue medication and medication considered essential to treat AEs, all medications (prescription and non-prescription), herbal remedies and nutritional supplements should be avoided from the time of first IMP administration until after Day 28 or completion of the course of antimalarial rescue medication (whichever is later). Incidental and limited use of medications not believed to affect subject safety nor 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 as described in the exclusion criteria of this protocol (see Section 4.2).

Due to possible liver stress and alanine aminotransferase (ALT) over aspartate aminotransferase (AST) elevations after malaria inoculation, ibuprofen (at doses of up to 1.8 g/day) is the preferred treatment over paracetamol for possible emergent malaria symptoms.

6.2 PROHIBITED CONCOMITANT THERAPIES

The following medications are prohibited during the study:

- Drugs with known antimalarial activity (trimethoprim sulfamethoxazole, tetracycline, doxycycline, erythromycin, clarithromycin, azithromycin, clindamycin, rifampicin, or newer quinolones, benzodiazepines, flunarizine, fluoxetine, methotrexate, chloroquine and hydroxychloroquine);
- Drugs known to cause drug-drug interactions with artemether-lumefantrine (Riamet®), atovaquone-proguanil (Malarone®) or primaquine (Primaquine®), including but not limited to cimetidine, metoclopramide or antacids;
- Any drug susceptible to interact with BCRP (cyclosporine, elacridar, eltrombopag, gefitinib).

7. ASSESSMENTS

7.1 TIMING OF ASSESSMENTS

An overview of the timing of PfSPZ DVI challenge, treatment and assessments is given in the Time and Events Schedule.

If assessments are planned at the same time point in the study, the order of assessments should be as follows:

1. 12-lead ECG recordings;
2. Vital signs measurements;
3. Blood sampling;
4. Other assessments.

The following time-related windows for PK sampling, body temperature, blood pressure, pulse rate, ECG, haematology, blood chemistry and urinalysis assessments will be acceptable based on logistical and operational considerations:

- Pre-IMP and pre-inoculation observations within 3 hours prior to dosing/inoculation;
- ± 5 min from 0.5 to 4 hours post dose (inclusive);
- ± 15 min from >4 to 12 hours post dose;
- ± 30 min >12 to <24 hours post dose (inclusive);
- ± 60 min for 24 hours post dose;
- ± 10% deviation from theoretical post dose times for >24 hours to <120 hours post dose;
- a deviation of ± 1 day for further days.

All assessments scheduled for a particular time point should be performed within these windows.

Any deviations from the above-mentioned window periods will be documented as protocol non-compliances and will be evaluated for significance at the data review meeting prior to database lock.

Adverse events and the intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity.

7.1.1 *Screening Period*

Screening

Screening for eligible and consenting subjects will be performed within approximately 4 weeks prior to randomisation.

Subjects will be given a full explanation of the nature of the study and written informed consent (approved by the local ethics committee) will be obtained according to local requirements before any study-related assessment will be carried out.

At screening, subjects will be asked to attend the clinical unit to have assessments performed as indicated in the Time and Events Schedule.

All results from the screening procedure needed to evaluate eligibility, including the clinical laboratory results, must be available prior to the first administration of the IMP on Day 1 for Cohort 1 and prior to PfSPZ Challenge DVI on Day 1 for Cohorts 2 and 3. Any abnormal assessment at the screening visit will be assessed according to its clinical relevance, and if found relevant, the subject will not be included in the study.

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 subject's eligibility, or in case of a positive urine drug screen. The result of the retest will be considered for subject eligibility. Findings made during unscheduled visits should be reported in the eSource system.

7.1.2 *Treatment Period*

Day -1

Subjects will be enrolled in one of the 3 cohorts. Subjects will be admitted to the clinical unit in the morning of Day -1. Subjects in Cohort 1 will remain at the clinical unit until Day 4, i.e. 36 hours after second IMP administration. Subjects in Cohorts 2 and 3 will remain at the clinical unit until Day 13, i.e. 12 days post PfSPZ Challenge.

Eligibility of the subjects will be confirmed and assessments will be performed as indicated in the Time and Events Schedule.

Dinner will be served and no food intake will be allowed thereafter until 2 hours after the first administration of the IMP on the following day (Day 1). Intake of water will be allowed until 1 hour before the first administration of IMP. Fasting conditions will be maintained for 2 hours, including drinking water that will be allowed from 2 hours post dose onwards (ad libitum but approximately 1500mL/24 hours).

Day 1

On Day 1, subjects in Cohorts 2 and 3 will be administered 3,200 *P. falciparum* sporozoites by DVI.

Subjects in all cohorts will be randomised in a 3:1 ratio, to receive two consecutive administrations of either P218 or placebo.

Subjects in Cohort 1 will receive a first dose of IMP; and so do the subjects in Cohorts 2 and 3, 2 hours after PfSPZ Challenge inoculation. For Cohorts 2 and 3, this initial dose at Day 1 must not be given prior to 2 hours post-inoculation.

Predose procedures will be performed as indicated in the Time and Events Schedule, as close to the time of IMP administration as possible and surely within 3 hours before dosing. Predose PK samples should be taken within 1 hour before IMP administration. After the predose procedures, the subjects will be asked to ingest the first dose of study drugs according to the procedure described in Section 5.6, followed by repeated blood sampling and other assessments as outlined in the Time and Events Schedule.

Day 2

Dinner will be served and no food intake will be allowed thereafter until 2 hours after the administration of the IMP on the following day (Day 3). Intake of water will be allowed until 1 hour before administration of IMP. Fasting conditions will be maintained for

2 hours, including drinking water that will be allowed from 2 hours post dose onwards (ad libitum but approximately 1500mL/24 hours).

Day 3

Subjects in all cohorts will receive a second dose of IMP on Day 3, i.e. 48 hours after first IMP administration.

Predose procedures will be performed as indicated in the Time and Events Schedule, as close to the time of IMP administration as possible and surely within 3 hours before dosing. Predose PK samples should be taken within 1 hour before IMP administration. After the predose procedures, the subjects will be asked to ingest the dose of study drugs according to the procedure described in Section 5.6, followed by repeated blood sampling and other assessments as outlined in the Time and Events Schedule.

7.1.3 *Follow-up Period*

After discharge from the clinical unit on Day 4, subjects in Cohort 1 will be followed up with daily ambulatory visits to the clinical unit up to Day 9. After discharge from the clinical unit on Day 13, subjects in Cohorts 2 and 3 will be followed up with daily ambulatory visits to the clinical unit until development of positive parasitaemia or until Day 28 otherwise.

The subjects in Cohorts 2 and 3 will receive rescue therapy (see Section 5.2) if positive parasitaemia is confirmed and will continue to be monitored daily at the clinical unit until treatment success. Upon treatment success, daily visits to the clinical unit are stopped, except for subjects that first need to complete the 3-day rescue treatment regimen and associated assessments. All subjects will be assessed again for parasitaemia at the EOS visit on Day 35. Subjects not developing positive parasitaemia until Day 28 will receive rescue therapy on that day. Instead of daily monitoring at the clinical unit until treatment success, these subjects will only be assessed again for parasitaemia at the EOS visit on Day 35.

Assessments will be performed as indicated in the Time and Events Schedule and in Section 5.2).

All subjects in Cohorts 2 and 3 will be assessed for parasitaemia at the EOS visit on Day 35. In order to provide some flexibility for the subjects regarding the site visit and to maintain the integrity of the study design, a time window of ±1 day is permitted, in case of time conflict or unforeseen circumstances. All subjects that receive rescue therapy will be asked non leading questions to determine the occurrence of any AEs approximately two weeks after initiation of the rescue treatment, either during a planned study visit or by phone in case there is no planned study visit at that time. If this time point is after the EOS visit on Day 35, the moment of the phone call will be considered the EOS and not Day 35. In order to provide some flexibility regarding the phone call and to maintain the integrity of the study design, a time window of ±2 days is permitted for this follow-up call, in case of time conflict or unforeseen circumstances.

7.1.4 *Unscheduled Visits*

Unscheduled visits can be planned for instance:

- to obtain additional information to ensure safety to the subject. Additional blood and urine samples may be taken at the discretion of the Investigator.
- to assess, confirm and follow-up on out-of-range clinical laboratory test, vital sign or ECG values that will determine a subject's eligibility, or in case of a positive drug screen. The result of the retest will be considered for subject eligibility.

Findings made during unscheduled visits should be reported in the eSource system.

7.2 PHARMACOKINETIC EVALUATIONS

7.2.1 *Sample Collection and Handling*

Blood samples

At each planned time point, a 4 mL venous blood sample will be drawn for analysis of P218 (and its metabolites in Cohort 1 only) in plasma, as described in the Time and Events Schedule. The exact date and time of blood sampling and of administration of the study drugs must be recorded in the eSource system.

Every blood sample will be drawn by venipuncture or via indwelling cannula in the forearm into a pre-chilled EDTA vacuum tube and immediately placed in an ice bath. Further processing of the sample must be completed within 30 minutes.

Detailed procedures for sample collection, shipment, processing and storage will be described in a laboratory manual. Briefly, the sample will be acidified with 10 µL/mL 2M citric acid and centrifuged under refrigeration, to isolate plasma. Two aliquots of at least 0.5 mL will be prepared in screw-cap polypropylene tubes and immediately frozen on dry ice and stored at -80 °C until analysis. Shipment of aliquots to Swiss Bioquant must take place on dry ice – only the first aliquot of each sample will be shipped, the second one must remain at the clinical unit and can be shipped by request in a separate parcel.

7.2.2 *Bioanalyses*

Plasma samples for determination of P218 concentration, and concentration of its major metabolites P218 β-acyl-glucuronide, P218-OH β-acyl-glucuronide and possibly P218-OH in Cohort 1 only, will be analysed by Swiss BioQuant laboratory on behalf of MMV using a validated high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method. Analytical methodology will be detailed in a separate Bioanalytical Study Plan. Outcomes of analyses will be reported in a dedicated Bioanalytical report which will be attached to or referred to in the Clinical Study Report upon completion of the study.

No human deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) analysis will be performed.

7.2.3 *Pharmacokinetic Parameters*

Pharmacokinetic calculations will be performed by SGS-Life Sciences using Phoenix WinNonLin 8.0 or higher (Pharsight Corporation, Palo Alto, CA, USA).

The following parameters, where appropriate, will be determined for P218, and in Cohort 1 only also for its major metabolites (P218-OH, P218-beta-acyl-glucuronide,

P218-beta-acyl-glucuronide-OH) using individual concentration data in plasma, according to the definitions and methods of calculation below:

- AUC_{τ} = $AUC_{0\text{-}48h}$ after each administration (i.e. $AUC_{0\text{-}48h}$ and $AUC_{48\text{-}96h}$);
- $AUC_{48h\text{-}inf,}$ calculated from $AUC_{48h\text{-}t} + (C_t/\lambda_z)$;
- $AUC_{48h\text{-}last}$;
- CL/F and Vz/F after the second administration (not for metabolites);
- C_{max} and t_{max} after each administration;
- $t_{1/2}$ after the second administration;
- R_{ac}, calculated as $AUC_{48\text{-}96h}/AUC_{0\text{-}48h}$;
- Metabolic ratio of AUC_{τ} and $AUC_{48h\text{-}inf}$ for P218 β-acyl glucuronide, P218-OH and P218-OH β-acyl glucuronide over parent P218 (Cohort 1 only).

Additional pharmacokinetic parameters may be calculated as appropriate.

7.3 EFFICACY EVALUATIONS

For an overview of efficacy endpoints, see Section 2.2.

7.3.1 *Parasitaemia*

A blood sample of maximum 1.5 ml will be collected via direct venepuncture in an EDTA tube from each subject at time points where parasitological assessments are scheduled Time and Events Schedule. The assessment of malaria parasitology by TBS and qPCR will be as follows:

- Parasite density, expressed as the number of parasites per microliter of blood will be measured using the routine diagnostic TBS microscopy method for parasite count employed by the centre of excellence for tropical medicine (ITM) as per relevant standard operating procedure and Laboratory Manual. The reading will be considered positive if 2 unambiguous malaria structures are seen in at least 0.5µl of blood and the observation is confirmed by a second expert malaria microscopist.
- *var*ATS (the acidic terminal segment in *Plasmodium var* genes) targeted qPCR assay of parasite load will be performed in accordance with the ITM standard operating procedure and the Laboratory Manual. Method validation and external quality assessment (EQA) of the outcomes will be carried out at the Department of Laboratory Medicine, School of Medicine, University of Washington (Dr. Sean Murphy) using 18S rRNA targeted qRT-PCR methodology described in a dedicated Validation Plan. Given the higher sensitivity of qPCR compared to microscopy, this method will be used to confirm aparasitaemia after definitive antimalarial therapy for all subjects. A subject will be considered cured following completion of the course of rescue antimalarial therapy and after qPCR results are obtained with values below the limit of detection.

The results of the TBS microscopy and qPCR at the ITM will be available in approximately 24 hours.

7.3.2 *Malaria Clinical Score*

An inoculum-related event is a sign or symptom associated with malaria infection (confirmed by a positive *P. falciparum* PCR [defined for the purpose of this study as ≥ 250 asexual parasites per mL] at the onset of the event) that is of expected intensity, frequency and duration for the individual subject in the context of this study.

Prevention of expected signs and symptoms associated with malaria infection form part of the efficacy evaluation of the study and are listed in Attachment 1. If the presence of *P. falciparum* malaria is confirmed by positive PCR at the time of onset of these signs/symptoms and if, in the Investigator's opinion, the signs/symptoms are of the expected intensity, frequency and duration for the individual subject in the context of this study, then the events will be classified as inoculum-related events and reported as such in the final clinical study report.

Inoculum-related events meeting the classification criteria for SAEs or AEs of special interest should be subjected to standard expedited reporting procedures of these events (See Section 10).

The following malaria signs/symptoms will not be classified as inoculum-related events:

- If the PCR results for *P. falciparum* are between 0 and < 250 asexual parasites per mL at the time of the onset of the event, usual AE/SAE reporting procedures and criteria will apply (see Section 10), and the event will not be classified as an inoculum-related event.
- Observed malaria symptoms or signs that are of greater intensity, frequency or duration than would be expected in the context of this study, will be regarded as medically important events and must be reported promptly (i.e. in an expedited manner) to the Sponsor using an SAE report form (see Section 10.7), and will not be classified as inoculum-related events.

Final classification of signs and symptoms as inoculum-related event or AE will occur after PCR results are available.

7.3.3 *PK/PD*

A model-based analysis is foreseen to characterise the PK/PD relationship between P218 plasma concentrations and blood stage asexual parasitaemia. Details of the modelling analysis will be described in the modelling analysis plan (MAP).

7.4 SAFETY EVALUATIONS

The safety assessment in this study will be based on AEs, clinical laboratory tests including serum folate, vital signs, ECG, physical examination and Beck depression Inventory (screening only) as described in the following sections.

7.4.1 *Adverse Events*

Adverse events will be monitored continuously from informed consent until the last study-related activity. At regular intervals during the study, subjects will be asked non-leading questions to determine the occurrence of any AEs. All AEs reported spontaneously during the course of the study will be recorded as well.

For detailed definitions and reporting procedures of AEs, see Section 10.

7.4.2 *Clinical Laboratory Tests*

Blood samples will be collected by venipuncture or via indwelling cannula at the time points indicated in the Time and Events Schedule. Biochemistry and haematology testing will be performed on these samples, as well as immunology testing (HbsAg, anti-HCV antibody and HIV antibody and antigen) on the sample from screening. In all female subjects, also serum β-HCG assessment at screening and urine β-HCG assessments on Day-1 and at the EOS visit will be performed. FSH will be measured at screening in all women.

All blood samples for safety assessments, except the ones taken during rescue treatment for Troponin I measurements only (see Section 5.2), should be taken in a fasted state (overnight fast for at least 8 hours for unbiased glucose determination).

Standard laboratory tests will be performed by ZNA Middelheim.

The following biochemistry and haematology tests will be performed on the safety blood samples:

- Biochemistry: sodium, potassium, chloride, bicarbonate, urate, inorganic phosphate, creatinine, albumin, glucose, AST, ALT, alkaline phosphatase (ALP), gamma glutamylaminotransferase (GGT), lactate dehydrogenase (LDH), total and direct bilirubin, total serum proteins, blood urea nitrogen (BUN), C-reactive protein (CRP), creatine phosphokinase (CPK) and troponin I (Cohorts 2 and 3 only);
- Haematology: haemoglobin, haematocrit, red blood cell (RBC) count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count, platelet count, reticulocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes;
- Coagulation: prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT).

Serum folate will be measured according to the time points in the Time and Events Schedule.

G6PD enzyme test will be performed at screening.

A midstream urine sample will be collected for urinalysis by dipstick for glucose, protein, nitrite, pH and occult blood. Microscopic examination for WBC, RBC and casts will be performed

A urine drug screen (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates) and an alcohol breath test will be performed at the time points indicated in the Time and Events Schedule.

The Investigator must review the laboratory report, document this review and record any change occurring during the study he/she considers to be clinically relevant in the eSource system. Laboratory values outside the normal range will be flagged and their clinical relevance will be assessed by the Investigator.

Samples that remain after protocol-specific assessments have been performed may be used for further exploratory work on pharmacokinetics, metabolites, plasma protein binding, protein analysis and biochemistry. No human DNA or RNA analysis will be performed.

7.4.3 *Vital Signs*

Vital sign parameters will be assessed after 5 minutes in supine position at the time points indicated in the Time and Events Schedule. The vital sign parameters that will be assessed are supine systolic and diastolic blood pressure (SBP and DBP, respectively), pulse rate and body temperature (sublingual). In Cohort 1, orthostatic changes to BP and pulse rate will also be assessed at screening: Subjects will be requested to stand after completion of the supine measurements and blood pressure and pulse rate will be recorded after 2 minutes in the standing position.

These parameters will be measured using a completely automated device consisting of an inflatable cuff and an oscillatory detection system. All values will be registered on a built-in recorder so that measurements are observer-independent.

Any change from baseline in vital sign values occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the eSource system.

7.4.4 *Electrocardiogram*

Twelve-lead ECGs recordings will be will be recorded after 10 minutes in supine position at the time points indicated in the Time and Events Schedule.

All recordings will be performed once, except at screening and before the first and second IMP administration when they will be performed in triplicates at approximately 1-minute intervals. Paper speed will be 25 mm/s, so that the different ECG intervals can be measured manually.

Between Day 10 and 28, one record will be performed on the first day of rescue treatment only.

The interpretations of the ECGs will be performed by the Investigator or his/her designee at the clinical unit. Any change from baseline ECG occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the eSource system.

7.4.5 *Physical Examination*

Physical examination will be performed at the time points indicated in the Time and Events Schedule.

Height is to be measured barefoot and at screening only. Body weight to be measured as indicated in the Time and Events Schedule. To obtain the actual body weight, subjects must be weighed lightly clothed at screening.

Any change in physical examination occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the eSource system.

After screening, a targeted, symptom-driven physical examination will be performed focused on changes since the previous examination, but will always include at least: general appearance, skin, heart/circulation, chest, lungs, abdomen and brief neurological examination.

7.4.6 *Beck Depression Inventory*

The Beck depression inventory is performed at screening only (see Attachment 2).

The questionnaire is scored by the subject. The inventory completed by the subject will be reviewed/checked for completeness and the total score calculated by the study personnel.

7.5 TOTAL VOLUME OF BLOOD SAMPLING

The total volume of blood from any single subject will not exceed the maximum allowable volume of 450 mL in any given 30-day period during his/her participation in the study.

If necessary, in order to obtain additional information to ensure a subject's safety, additional blood samples (up to 50 mL) and/or urine samples may be taken at the discretion of the Investigator.

7.6 APPROPRIATENESS OF MEASUREMENTS

The assessments which will be made in this study are standard, and are generally recognized as reliable, accurate and relevant.

8. STUDY TERMINATION/COMPLETION

8.1 STUDY COMPLETION

A subject will be considered to have completed the study if he or she has completed the required EOS visit and EOS phone call.

8.2 REMOVAL OF SUBJECTS FROM THERAPY OR STUDY

Subjects have the right to withdraw from the study at any time for any reason, including personal reasons. A subject can withdraw without giving a reason. This will not affect his/her future care. The Investigator should however try to find out why a subject withdraws from the study and document the reason for withdrawal in the source documents and on the in the eSource system.

All withdrawn subjects who were inoculated with the PfSPZ challenge agent must receive antimalarial rescue therapy as described in Section 5.2. Subjects who fail to return for visits will be traced as described in Section 8.4.

Subjects **may** be withdrawn in the event of:

- A severe AE or SAE.

Subjects **must** be withdrawn in the event of:

- Withdrawal of consent;
- For safety reasons, it being in the best interest of the subject that he/she be withdrawn, in the Investigator's opinion or in the opinion of the SRT;
- A positive pregnancy test (the subject or, in the case of a male subject, his female partner), or if the subject/partner is non-compliant with the contraception requirements (see Section 4.1);
- Development of a medical condition that requires concomitant treatment with a prohibited therapy (see Section 6.2);
- Failure of the subject to comply with the protocol requirements or to cooperate with the Investigator resulting in a significant risk to the subject's safety;
- Breaking of the randomisation code during administration of the IMPs, by the Investigator or by a member of his/her clinical staff. If the code is broken by the Sponsor, for safety reporting purposes, the subject may remain in the study.

In the event of a subject being withdrawn, the monitor and Sponsor should be informed: in case of withdrawal due to an SAE (for details on AE reporting see Section 10), the Sponsor should be notified within 24 hours; in case of withdrawal for other reasons; the Sponsor should be notified within 2 days from the event.

If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until satisfactory health has returned.

Subjects who are withdrawn from the study prior to completion of the scheduled study procedures for any reason (AE, withdrawal of consent, etc.) should be invited to complete the assessments as much as possible: as long as the subject consents, all relevant assessments of the day on which the subject withdrew from the study should be completed, at least those related to safety, and the subject should come for a safety

follow-up visit 4 weeks after the last administration of IMP. In case of an AE, the appropriate follow-up will be done.

Subjects who are withdrawn from the study for reasons other than safety may be replaced. This decision will be made based upon discussion and mutual agreement between the Sponsor and the Investigator.
IMPs that are assigned to a subject that withdraws must not be assigned to another subject.

8.3 STOPPING RULES OR DISCONTINUATION CRITERIA

The study will be overseen by an SRT (see Section 5.8).

8.4 TRACEABILITY OF SUBJECTS

The following measures will be taken to assure traceability of subjects in Cohorts 2 and 3:

- Following scheduled, in-house confinement within the pharmacology unit or removal from the study, all subjects will be contacted daily until Day 28.
- All subjects will commit to remaining contactable throughout the study and this will be recorded on the ICF.
- Subjects will be required to provide current contact details (two telephone numbers including a mobile number and a responsible adult as an emergency contact) and a relevant email address.
- Should a subject not be contactable by the unit, all alternative methods possible (e.g. sending someone to home address of subject) will be used to locate and communicate with the subject.
- Should a subject still be uncontactable the situation will be discussed with the Sponsor and escalated as appropriate including alerting the regulatory authorities to a deviation from protocol.

9. STATISTICAL METHODS

9.1 STATISTICAL ANALYSIS

All statistical analysis will be performed by SGS Life Sciences, using SAS® (SAS Institute Inc., Cary, NC, USA; version 9.4 or higher) software for statistical computations.

The standard descriptive statistics for continuous variables are the number of subjects (N), mean, standard deviation (SD), median, first and third quartiles, minimum and maximum values. The standard descriptive statistics for categorical variables are the number of subjects in the category and the proportion expressed as a percentage.

Fasting placebo data from the Cohorts 1, 2 and 3 will be pooled while IMP treatments per cohort are considered as two different treatment groups.

9.1.1 *Planned Analyses*

The primary objective of this analysis is the assessment of the chemoprotective activity of P218 in *P. falciparum* CHMI in non-immune healthy volunteers after PfSPZ Challenge through DVI.

No interim analysis will be performed.

All statistical methods shall be detailed in a Statistical Analysis Plan (SAP) that will be finalized before database lock.

9.1.2 *Analysis Populations*

The following populations will be considered for analysis:

- Intent-to-treat (ITT) population, defined as all randomised subjects who received at least one dose of IMP and who received the PfSPZ challenge inoculation, analysed as randomised;
- Safety (SAF) population, defined as all subjects who received at least one dose of IMP, analysed as treated;
- Pharmacokinetic (PK) population, defined as all subjects who have received at least 1 dose of IMP and had measurable concentrations of parent and/or metabolites;
- Efficacy population, defined as all subjects in the safety population who received PfSPZ Challenge by DVI;
- PK/PD population, defined as all subjects belonging to both the PK and the efficacy population.

The ITT population will be used for the analysis of demographics and efficacy, the pharmacokinetic population will be used for the pharmacokinetic statistical analysis, the efficacy population for the efficacy statistical analysis and the safety population will be used for safety/tolerability analysis.

9.1.3 *Initial Characteristics Data of the Subject Sample*

For all randomised and treated subjects, descriptive statistics will be provided per treatment group for demographic (e.g. age, height, weight, BMI, race, gender) and other

initial subject characteristics (alcohol and drug screening tests, pregnancy test, orthostatic changes to blood pressure and pulse rate [Cohort 1 only], G6PD enzyme test, serology, medical and social history, concomitant diseases, Beck Depression Inventory).

Prior and concomitant medications will be coded using the World Health Organization (WHO)_DRUG Dictionary.

9.1.4 *Pharmacokinetic Data*

Pharmacokinetic concentrations will be summarized by treatment group, day and scheduled sampling times by using number of subjects with data, arithmetic mean, standard deviation, coefficient of variation, median, minimum and maximum.

For PK parameters (see Section 7.2.3), descriptive statistics will be included with in addition geometric mean and geometric coefficient of variance (CV%); t_{max} will be summarized by using number of subjects with data, median, minimum and maximum.

Individual and mean concentrations versus time figures will be presented.

9.1.5 *Efficacy Data*

9.1.5.1 *PARASITAEMIA*

Primary endpoint: The duration from PfSPZ challenge DVI to positive parasitaemia will be analysed using descriptive statistics, including the geometric mean and corresponding two-sided 90% confidence intervals. In the absence of positive parasitaemia, the duration will be set to a maximum of 28 days.

The number and proportion of subjects with presence of positive parasitaemia between inoculation with PfSPZ and Day 28 (or the administation of rescue medication) will be summarized by a responder analysis per treatment group. Corresponding two-sided 90% Exact Clopper-Pearson confidence limits will be presented as well.

9.1.5.2 *MALARIA CLINICAL SCORE*

For the malaria clinical score that will be administered by the PI or study physician, actual values and changes from baseline will be evaluated by means of descriptive statistics. Additionally, expected signs and symptoms will be summarized by score.

9.1.6 *Safety Data*

Safety parameters will be tabulated and analysed descriptively.

9.1.6.1 *ADVERSE EVENTS*

The original terms entered in the eSource system by Investigators to identify AEs will be fully described and coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

The reported AEs will be allocated to phases based on their start date. All AEs will be listed. For Cohort 1 all AEs with onset after first IMP treatment will be summarized by treatment group. For Cohorts 2 and 3 all AEs with onset after PfSPZ challenge DVI will be summarised by treatment group. Summaries will be made per MedDRA primary system organ class, MedDRA preferred term, severity, with the number and percentage

of subjects and the number of events. Similar summaries will be prepared for AEs considered to be related to IMP and AEs considered to be related to the PfSPZ challenge DVI, for serious AEs and AEs of special interest.

Special attention will be paid to those subjects who died, discontinued IMP due to an AE, or experienced a severe or serious AE. Summaries, listings and narratives (also see Section 12.11) may be provided, as appropriate.

9.1.6.2 *CLINICAL LABORATORY TESTS*

Serum folate, continuous biochemistry and hematology laboratory tests will be evaluated by means of descriptive statistics on the actual values, at each assessment time point and by treatment group. Changes from baseline will also be summarized using descriptive statistics by assessment time point and by treatment group.

Relative changes in clinical laboratory test values compared to values at baseline will be evaluated in accordance with the normal ranges of the clinical laboratory (below, within or above normal range). The percentage of subjects with clinical laboratory test abnormalities will be summarized by treatment group.

The number and percentage of subjects with liver enzyme elevations after IMP administration as defined below will be summarised:

- ALT or AST >3 x Upper Limit of Normal (ULN);
- ALT or AST >5 x ULN;
- ALT or AST >8 x ULN;
- ALT or AST >3 x ULN and bilirubin >2 x ULN at the same time point, together with a conjugated bilirubin fraction> 35% (Potential Hy's law cases).

A listing of subjects with any clinical laboratory test result outside the reference ranges will be provided.

9.1.6.3 *VITAL SIGNS*

Vital signs parameters will be assessed after 5 minutes in supine position at the time points indicated in the Time and Events Schedule. Pulse rate, systolic blood pressure, diastolic blood pressure and body temperature will be evaluated by means of descriptive statistics (actual values and changes from baseline).

The percentage of subjects with vital signs abnormalities will be summarized by treatment group in a cross-tabulation of post-baseline versus baseline abnormalities to the normal ranges (as defined in Attachment 3).

9.1.6.4 *ELECTROCARDIOGRAM*

12-lead ECG recordings will be performed after subjects remained in a supine position for at least 10 minutes. All recordings will be performed once, except at the screening and before the first and second IMP administration when they will be performed in triplicates.

All ECG data automatically measured by ECG devices (PR, QRS, QT, QTcB, QTcF and HR) and overall ECG evaluation will be listed. The ECG data, along with changes from

baseline will be summarised by means of descriptive statistics at each assessment time point and by treatment group.

The percentage of subjects with ECG abnormalities will be summarized by treatment group in a cross-tabulation of post-baseline versus baseline abnormalities to the normal ranges (as defined in Attachment 3).This cross-tabulation will include categorical assessment on actual values and changes from baseline of QTcB and QTcF prolongation.

9.1.6.5 *PHYSICAL EXAMINATION*

Abnormal findings in physical examination will be listed.

9.2 DETERMINATION OF SAMPLE SIZE

Thirty-two subjects will be enrolled in 3 cohorts of 8, 12 and 12 subjects. In agreement with the Sponsor, additional subjects may be recruited in each cohort, to replace discontinuations for non-safety reasons and achieve cohort sizes of 8 and 12.

This is an exploratory study thus no sample size calculation is performed. However, if the nine treated subjects each in Cohort 2 and 3 do not develop positive parasitaemia (qPCR $\geq$ 250 asexual parasites per mL) after IMP administration and until Day 28, it may be concluded that the protection rate for P218 is 0.72 (72%) with a 95% probability (lower limit of exact, one sided test, 95%, CI 0.72). This holds true for both Cohort 2 and Cohort 3.

10. ADVERSE EVENT REPORTING

10.1 DEFINITIONS

Adverse Event

An AE is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal finding), symptom or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product.

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal result of diagnostic procedures, including clinical laboratory test abnormalities.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose meets any of the following conditions:

- Results in death;
- Is life-threatening, i.e. the subject was at risk of death at the time of the event (e.g. ventricular fibrillation and anaphylaxis). The term does not refer to an event which hypothetically might have caused death if it were more severe.
- Requires inpatient hospitalization or prolongation of existing inpatient hospitalization:
  Hospitalization refers to an overnight admission into hospital for the purpose of investigating and/or treating the AE. Hospitalization for an elective procedure, or routinely scheduled treatment for a pre-existing condition that is unrelated to the study and has not worsened, is not an SAE. Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of an SAE given above and not resulting in hospital admission, is not an SAE. Cosmetic surgery or for social reasons or respite care in the absence of any deterioration in the subject's general condition, is not an SAE;
- Results in persistent or significant disability/incapacity, i.e. causing substantial disruption of the subject's ability to conduct normal life;
- Is a congenital anomaly/birth defect;
- Is medically significant, i.e. may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject's health or may require intervention to prevent one of the above outcomes. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization or development of drug dependency or drug abuse.
- Constitutes a possible Hy's Law case (defined as a subject with any value of ALT or AST greater than or equal to 3xULN together with an increase in bilirubin to a value greater than 2xULN [>35% direct] and NOT associated with an ALP value greater than 2xULN.)

Adverse Events of Special Interest (AESIs)

An adverse events of special interest (AESIs) (serious or non-serious) is of scientific and medical concern specific to the Sponsor's product or programme, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor could be appropriate. Such an event might require further investigation in order to characterise and understand it. Depending on the nature of the event, rapid communication by the study Sponsor to other parties (e.g., regulators) might also be warranted (CIOMS VI, ICH E2F, 2010). For the purpose of this study, any abnormalities listed below should be reported as AESI:

Hepatic

- Any ALT or AST above 3x ULN;
- Any elevation in bilirubin 2x ULN;
- Any AST or ALT above 2x ULN and (total bilirubin level (TBL) >1.5x ULN or INR >1.4);
- Any AST or ALT above 2x 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).

Cardiac

- QTcB or QTcF at any time >480 ms;
- 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.

Haematological

- Haemoglobin drop >2 g/dL and under lower limit of normal from baseline prior to inoculation;
- Absolute neutrophil count <1000/µL;
- Platelet count <100,000 /mm^3.

Dermatological:*

- Any suspected cutaneous adverse event, e.g., rash

* if one of these cutaneous reaction is observed and when feasible, pictures of the lesions should be obtained.

Unlisted (Unexpected) Adverse Event

An AE is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information (Investigator's Brochure for drugs in clinical development or approved manufacturer's prescribing information for marketed drugs). For this protocol, the reference documents for the assessment of expectedness for P218 and PfSPZ Challenge are the Investigator's Brochures for each of these products [6,7]. Expected reactions to the antimalarial rescue treatment are described in the product's prescribing information:

- P218 (DHFR inhibitor) side-effects may include bone marrow suppression (rarely), GI symptoms including nausea and vomiting and skin rashes. All of these are reversible by the addition of folates to the subject's diet.
- Malarial challenge (sporozoites) can be associated with itching, pain and swelling at the site of inoculation. This usually resolves within 24-48 hours.
- Riamet (antimalarial/EOS treatment: artemether and lumefantrine) may be associated with the following adverse events; these may occur with a varying periodicity:

Frequency of Undesirable Effects

	Adults and adolescents above 12 years of age
Immune system disorders	
Hypersensitivity	Not known
Metabolism and nutrition disorders	
Decreased appetite	Very common
Psychiatric disorders	
Sleep disorders	Very common
Insomnia	Common
Nervous system disorders	
Headache	Very common
Dizziness	Very common
Paraesthesia	Common
Ataxia, hypoaesthesia	Uncommon
Somnolence	Uncommon
Clonus	Common
Cardiac disorders	
Palpitations	Very common
Electrocardiogram QT prolonged	Common
Respiratory, thoracic and mediastinal disorders	
Cough	Common
Gastrointestinal disorders	
Vomiting	Very common
Abdominal pain	Very common
Nausea	Very common
Diarrhoea	Common
Hepatobiliary disorders	
Liver function tests increased	Uncommon
Skin and subcutaneous tissue disorders	
Rash	Common
Pruritus	Common
Urticaria	Uncommon
Angioedema*	Not known
Musculoskeletal and connective tissue disorders	
Arthralgia	Very common
Myalgia	Very common
General disorders and administration site conditions	
Asthenia	Very common
Fatigue	Very common
Gait disturbance	Common

- Malarone (rescue medication: atovaquone and proguanil hydrochloride) may be associated with the following adverse events; these may occur with a varying periodicity:

System Organ Class	Very Common	Common	Uncommon	Rare	Not known[2]
Blood and lymphatic disorders		Anaemia Neutropenia [1]			Pancytopenia
Immune system disorders		Allergic reactions			Angioedema[3] Anaphylaxis (see section 4.4) Vasculitis[3]
Metabolism and nutrition disorders		Hyponatraemia[1] Anorexia	Elevated amylase levels[1]		
Psychiatric disorders		Abnormal dreams Depression	Anxiety	Hallucinations	Panic attack Crying Nightmares Psychotic disorder
Nervous system disorders	Headache	Insomnia Dizziness			Seizure
Cardiac disorders			Palpitations		Tachycardia
Gastrointestinal disorders	Nausea[1] Vomiting Diarrhoea Abdominal pain		Stomatitis		Gastric intolerance[3] Oral ulceration[3]
Hepatobiliary disorders		Elevated liver enzymes[1]			Hepatitis Cholestasis[3]
Skin and subcutaneous tissue disorders		Pruritus Rash	Hair loss Urticaria		Stevens-Johnson Syndrome Erythema multiforme Blister Skin exfoliation Photosensitivity reactions
General disorders and administration site conditions		Fever			
Respiratory, thoracic and mediastinal disorders		Cough			

An **inoculum-related event** is a sign or symptom associated with malaria infection (confirmed by a positive *P. falciparum PCR* at the onset of the event) that is of expected intensity, frequency and duration for the individual subject in the context of this study. Prevention of expected signs and symptoms associated with malaria infection form part

of the efficacy evaluation of the study and thus, inoculum-related events are not reported as AE (see Section 7.3.2 and Section 9.1.5.2).

A **rescue treatment-related AE** is a sign or symptom associated with the antimalarial rescue drug that is of expected intensity, frequency and duration for the individual subject in the context of this study. Expected reactions to the antimalarial rescue treatment are described in the product's prescribing information.

10.2 INTENSITY OF ADVERSE EVENTS

Each AE must be rated on a 5-point scale of increasing intensity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0:

Note: the semi-colon within the description of the grade indicates 'or'.

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 life (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).

Grade 3:

Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated, disabling; limiting self-care activities of daily life (bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden).

Grade 4:

Life-threatening consequences; urgent intervention indicated.

Grade 5:

Death related to AE.

10.3 CAUSALITY ASSESSMENT

The Investigator must assess the relationship of each event to the IMP, the PfSPZ inoculum and the rescue treatments (separately) and decide whether, in his or her medical judgement, there is a reasonable possibility that the event may have been caused by any of the study agents. Where possible, a distinction should be made between events considered related to the IMP, the PfSPZ inoculum and the rescue treatments. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Alternatively, if there is any valid reason for suspecting a possible cause-and-effect relationship between the investigational product(s) and the occurrence of the AE (even if undetermined or untested), then the AE should be considered as "related" to whichever product as relevant. This should be documented in the subject's source document and eSource system.

The following may guide this assessment:

- Related/suspected – the temporal relationship between the event and the administration of the IMP and/or PfSPZ inoculum is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies or accident;
- Not related/not suspected – the event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy or accident and no plausible temporal or biologic relationship exists between the IMP and/or PfSPZ inoculum and the event.

In addition to the assessments of relationship to the investigational products, the Investigator should comment on the adverse event record in the eSource system whether an adverse event is related to the study participation of the subject (study procedures etc.).

Of note, a sign or symptom associated with malaria infection (confirmed by a positive *P. falciparum* PCR at the onset of the event) that is of expected intensity, frequency and duration for the individual subject in the context of this study is considered to be an inoculum-related event. Prevention of expected signs and symptoms associated with malaria infection form part of the efficacy evaluation of the study and thus, inoculum-related events are not reported as AE (see Section 7.3.2).

10.4 ACTION TAKEN REGARDING THE IMP

The action taken towards the IMP must be described as follows:

- Permanently discontinued;
- No action taken;
- Unknown/Not applicable.

10.5 OUTCOME

The outcome of each AE must be rated as follows:

- Recovered/resolved;
- Recovering/resolving;
- Not recovered/not resolved;
- Recovered with sequelae/resolved with sequelae;
- Fatal;
- Unknown.

10.6 RECORDING OF ADVERSE EVENTS

All (S)AEs occurring during the clinical investigation must be documented in the eSource System.

Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g. cough, runny nose, sneezing, sore throat and head congestion should be reported as "upper respiratory infection"). Investigators must record their opinion concerning the relationship of the (S)AE to the study drugs in the eSource System. All measures required for (S)AE management must be recorded in the source documents and reported according to Sponsor's instructions.

All AEs occurring at any time during the study (including the follow-up period) will be followed by the Investigator until satisfactory resolution (e.g. value back to baseline value) or stabilization or until final database lock. If necessary, in order to obtain additional information to ensure safety to the subject, additional blood and urine samples may be taken at the discretion of the Investigator. Certain long-term AEs related to therapy cannot be followed until resolution within the setting of this study. In these cases follow-up will be the responsibility of the treating physician.

10.7 REPORTING OF SERIOUS ADVERSE EVENTS TO PRIMEVIGILANCE LTD.

All SAEs independent of the circumstances or suspected cause must be reported on a Serious Adverse Event Form by the Investigator to PrimeVigilance Ltd. within 24 hours of their knowledge of the event, preferably by fax (+44 800 471 5694) or by e-mail (MMV@primevigilance.com).

The SAE form should include a clearly written narrative describing signs, symptoms and treatment of the event, diagnostic procedures, as well as any relevant laboratory data and any sequelae, in order to allow a complete medical assessment of the case and independent determination of the possible causality.

Follow-up and outcomes should be reported for all subjects who experience an SAE.

It is critical that the information provided on the Serious Adverse Event Form matches the information recorded in the source documents and in the eSource system for the same event.

Copies of additional laboratory tests, consultation reports, postmortem reports, hospital case reports, autopsy reports and other documents should be sent when requested and applicable. Follow-up reports relative to the subject's subsequent course must be submitted to PrimeVigilance Ltd. until the event has subsided or, in case of permanent impairment, until the condition stabilizes.

10.8 PREGNANCY

All initial reports of pregnancy in subjects or in partners of male subjects must be reported by the Investigator to PrimeVigilance Ltd. within 24 hours of his/her knowledge of the event using a Pregnancy Form. Any subject who becomes pregnant during the study must be promptly withdrawn from the study (cfr. Section 8).

The Investigator will contact the subject at the expected time of delivery for follow-up. Abnormal pregnancy outcomes (e.g. spontaneous or induced abortion, stillbirth, neonatal death, congenital abnormality, birth defect) are considered SAEs and must be reported using the Serious Adverse Event Form.

10.9 REPORTING OF SERIOUS ADVERSE EVENTS TO COMPETENT AUTHORITIES/ETHICS COMMITTEES

PrimeVigilance Ltd. assumes responsibility for appropriate reporting of AEs to the regulatory authorities. PrimeVigilance Ltd. will also report to the Investigator all SAEs that are unlisted (unexpected) and associated with the use of the drug. The Investigator (or PrimeVigilance Ltd. where required) must report these events to the appropriate

Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol, unless otherwise required and documented by the IEC/IRB.

Adverse events reporting, including suspected unexpected serious adverse reactions (SUSARs), will be carried out in accordance with applicable local regulations.

After termination of the clinical study (determined as LPLV), any unexpected safety issue that changes the risks benefit analysis and is likely to have an impact on the subjects who have participated in the study, together with proposed actions, will be reported by the Sponsor/PrimeVigilance Ltd. to the competent authority(ies) concerned as soon as possible.

11. ETHICAL ASPECTS

11.1 STUDY-SPECIFIC DESIGN CONSIDERATIONS

Potential subjects will be fully informed of the nature of the study and of the risks and requirements of the study before any study-related assessment will be carried out. During the study, subjects will be given any new information that may affect their decision to continue participation. They will be informed that their participation in the study is voluntary and that they may withdraw from the study at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits and potential AEs of the study, and who provide their consent voluntarily will be enrolled in the study.

11.2 REGULATORY ETHICS COMPLIANCE

11.2.1 *Investigator Responsibilities*

The Investigator(s) should be qualified by education, training and experience to assume responsibility for the proper conduct of the study, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae or other relevant documentation requested by the Sponsor, the IRB/IEC or the regulatory authority(ies).

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines on Good Clinical Practice (GCP) and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of study subjects are protected, consistent with the principles originating from the Declaration of Helsinki (1964 and revisions), and that the clinical study data are credible.

11.2.2 *Independent Ethics Committee or Institutional Review Board (IEC/IRB)*

An IRB/IEC should safeguard the rights, safety and well-being of all study subjects. Special attention should be paid to studies that may include vulnerable subjects.

Before the start of the study, the Investigator (or Sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents:

- Final protocol and, if applicable, amendments;
- Sponsor-approved Informed Consent Form (ICF) (and any updates or any other written materials to be provided to the subjects);
- Sponsor-approved subject recruiting materials;
- Investigator Brochure (or equivalent information) and addenda;
- Available safety information;

- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable;
- Investigator's current curriculum vitae or other documentation evidencing qualifications (unless not required, as documented by the IEC/IRB);
- Information regarding funding, name of the Sponsor, institutional affiliations, other potential conflicts of interest and incentives for subjects;
- Any other documents that the IEC/IRB may require to fulfil its obligation.

This study will be undertaken only after the IEC/IRB has given full written approval of the final protocol and amendments (if any), the ICF(s) and updates (if any), applicable recruiting materials and any other written information to be provided to the subjects, and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study, the Investigator (or Sponsor where required) will send the following documents and updates to the IEC/IRB for its review and approval, where appropriate:

- Protocol amendments;
- Revision(s) to the ICF and any other written materials to be provided to the subjects;
- New or revised subject recruiting materials approved by the Sponsor;
- Revisions to compensation for study-related injuries or payment to subjects for participation in the study;
- Investigator's Brochure addenda or new edition(s);
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually);
- Reports of AEs that are serious, unlisted and associated with the IMP;
- New information that may adversely affect the safety of the subjects or the conduct of the study;
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects;
- Report of death of any subjects under the Investigator's care;
- Notification if a new Investigator is responsible for the study at the clinical unit;
- Development Safety Update Report, Short-Term Study Specific Safety Summary and Line Listings, where applicable;
- Any other requirements of the IEC/IRB.

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s), except when necessary to eliminate immediate hazard to the study subjects. If a deviation from or a change to the protocol was implemented to eliminate an immediate hazard to study subjects, then the implemented deviation or change, the reasons for it, and, if appropriate, the protocol amendment should be submitted to the IEC/IRB as soon as possible.

The Investigator (or Sponsor where required) will notify the IEC/IRB about the study completion within 90 days after the end of the study (defined as LPLV).

11.2.3 *Informed Consent*

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by both the Sponsor and the reviewing IEC/IRB. The informed consent should be in accordance with the principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements and Sponsor policy.

Before enrolment in the study, the Investigator or an authorized member of the clinical staff must explain to potential subjects the aims, methods, reasonably anticipated benefits and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may refuse to participate or withdraw consent to participate at any time, without penalty or loss of benefits to which the subject was entitled. Finally, they will be told that the Investigator will maintain a subject identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities and authorized Sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access, and agrees to allow his or her study physician to recontact the subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The language about the study used in the oral and written information, including the ICF, should be non-technical and practical and should be understandable to the subject (or the subject's legally acceptable representative). The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained consent, a copy of the ICF must be given to the subject.

If a subject (or legally acceptable representative) is unable to read or write, an impartial witness should be present during the entire informed consent discussion. After the written ICF and any other written information to be provided to the subjects, is read and explained to the subject (or legally acceptable representative), and after the subject (or legally acceptable representative) has orally consented to the subject's participation in the study and, if capable of doing so, has personally dated and signed the ICF, the witness should personally date and sign the consent form. By signing the ICF, the witness attests that the information in the ICF and any other written information was accurately explained to, and apparently understood by, the subject (or legally acceptable representative), and that informed consent was freely given by the subject (or legally acceptable representative).

11.2.4 *Privacy of Personal Data*

The collection and processing of personal data from subjects enrolled in the study will be limited to those data that are necessary to investigate the safety, quality and utility of the IMP used in the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal

data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data need to agree to keep the identity of the study subjects confidential.

The informed consent obtained from the subjects includes explicit consent for the processing of personal data and for the Investigator to allow direct access to subjects' original medical records for study-related monitoring, audit, IEC/IRB review and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

12. ADMINISTRATIVE REQUIREMENTS

12.1 PROTOCOL AMENDMENTS

Neither the Investigator nor the Sponsor will modify this protocol without a formal amendment. All protocol amendments must be issued by the Sponsor and signed and dated by the Investigator. Protocol amendments must not be implemented without prior IEC/IRB approval nor when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazard to the subjects, in which case an amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the Investigator and IEC/IRB must be provided to the Sponsor or his designee. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

12.2 SUBJECT IDENTIFICATION, ENROLMENT AND SCREENING LOGS

The Investigator agrees to complete a subject identification and enrolment log to permit easy identification of each subject during and after the study. This document will be reviewed by the Sponsor site contact for completeness.

The subject identification and enrolment log will be treated as confidential and will be filed by the Investigator in the study file. To ensure subject confidentiality, no copies will be made. All reports and communications related to the study will identify subjects by initials and/or assigned number only.

The Investigator must also complete a subject screening log which reports on all subjects who were seen to determine eligibility for inclusion in the study.

12.3 SOURCE DOCUMENTATION

The LabPas system is an electronic data capturing and information management system that will also serve as an eSource system for this study. The system combines all aspects of source data capturing with process control and clinical study management. All clinical and laboratory data, except those that are paper-based, will be collected directly in LabPas. The Source Document Identification Overview will specify which information will be eSource and which will be paper-based. The monitor will check data at the monitoring visits to the clinical unit. The Investigator will ensure that the data collected are accurate, complete and legible. Data will be monitored within LabPas by the study monitor who has only reading rights. Any changes required following monitoring will be made by site personnel or the Investigator and will be documented with a full audit trail within LabPas.

At a minimum, source documentation must be available for the following: subject identification, eligibility and study identification; date of informed consent, dates of visits, results of safety and efficacy parameters as required by the protocol, record of all AEs, follow-up of AEs, concomitant medication, drug receipt/dispensing/return records, IMP administration information, laboratory and ECG printouts (if not available digitally), date of study completion and reason for early discontinuation of IMP or withdrawal from the study, if applicable.

It is recommended that the author of an entry in the (e)Source documents be identifiable.

Source data may be directly captured from devices, transferred from third parties (e.g. laboratory data), or entered manually into the eSource system in use at the clinical unit. In such case, the majority of the source data will only be available electronically. The remainder of the data, captured initially on paper, may be entered retrospectively into the eSource system.

Following the ICH-GCP guidelines, direct access to (e)Source documentation (medical records) must be allowed.

12.4 CASE REPORT FORM COMPLETION

All source data, except those that are paper-based, will be collected directly into the eSource system. Paper-based source data will be manually transcribed to the eSource system. Only the data required for the clinical database will be transferred electronically from the eSource system to the clinical database.

12.5 MONITORING

Medical and clinical monitoring of the study will be done under the responsibility of the Sponsor by ICON Clinical Research and Biologic LLP, respectively.

The monitor will perform on-site monitoring visits as frequently as necessary. The monitor will record the dates of the visits in a study unit visit log that will be kept at the clinical unit. The first post-initiation visit will be made as soon as possible after enrolment has begun. At these visits, the monitor will compare the data captured in the eSource system for completeness and accuracy and perform data source verification to any data that has been captured as paper source or entered in the system later on. The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eSource system are known to the Sponsor and clinical staff and are accessible for verification by the Sponsor site contact. If electronic records are maintained at the investigational site, the method of verification must be discussed with the clinical staff.

Direct access to eSource documentation (medical records) must be allowed at all times for the purpose of verifying that the data recorded in the eSource system are consistent with the source documentation data. Findings from this review of captured data will be discussed with the clinical staff. During on-site monitoring visits (notified and agreed in advance with the clinical staff), the relevant clinical staff will be available, the eSource documentation will be accessible, and a suitable environment for review of study-related documents will be provided. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct.

12.6 DATA MANAGEMENT

Data management of the study will be performed under Sponsor delegation by SGS Life Sciences.

After the data entered in the eSource system are released by the Investigator, the data will be uploaded into the clinical database to perform cleaning activities. Computerized data cleaning checks will be used in addition to manual review, including listings review, to

check for discrepancies and to ensure consistency and completeness of the data. Queries emerging during data cleaning will be generated by the clinical data manager in the eSource system. The Investigator or his designee will answer the queries and update the source data, if needed.

The clinical database will be locked as soon as it is considered clean. Before the clinical database will be locked, the study eSource system will be locked by the clinical staff. Only authorized and well-documented updates to the study data are possible after database lock. The locked database is used in the final statistical analysis for study reporting. Measures will be undertaken to protect subject data handed over by the Investigator to the data management department and during inspections against disclosure to unauthorized third parties. Subject confidentiality will be maintained at all times.

12.7 DATA QUALITY ASSURANCE

The accuracy and reliability of the study data will be assured by the selection of qualified Investigators and appropriate study sites, review of protocol procedures with the Investigator and associated personnel prior to the study, and by periodic monitoring visits by the Sponsor or designate.

The Sponsor or his designee will review the eSource system for accuracy and completeness during (on-site) monitoring visits and after transmission to the Sponsor; any discrepancies will be resolved with the Investigator or designee, as appropriate. After upload of the data into the clinical study database, their accuracy verified using appropriate validation programs.

In accordance with Good Clinical Research Practice Guidelines and Recommendations, the Sponsor will be entitled to audit the facilities used in the clinical and laboratory parts of the study, as well as to access all the data files pertaining to the study. Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

12.8 ON-SITE AUDITS

Representatives of the Sponsor’s quality assurance department may visit the clinical unit at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the eSource system. Subject privacy must, however, be respected. The Investigator and clinical staff are to be present and available for consultation during routinely scheduled site audit visits conducted by the Sponsor or his designee.

Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

12.9 STUDY TERMINATION

The Sponsor has the right to terminate the study at any time. In case of an early termination of the study for safety reasons, or temporary halt by the Sponsor, the IEC/IRB and competent authorities should be notified within 15 calendar days and should be provided with a detailed written explanation for the termination/halt.

An end-of-study declaration will be submitted to the regulatory authorities and IEC/IRB after the complete study has ended. This notification will be submitted within 90 days after the end of the study.

12.10 RECORD RETENTION

In compliance with the ICH/GCP guidelines, the Investigator/Institution will maintain all eSource and all paper source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial and all study documents as specified by the applicable regulatory requirement(s). The Investigator/Institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the IMP. These documents will be retained for a longer period if required according to the applicable regulatory requirements or per agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained.

If the responsible Investigator retires, relocates, or for any other reasons withdraws from his responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the Investigator relocate or dispose of any study documents without having obtained written approval from the Sponsor.

If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation related to the study, the Investigator must permit access to such reports.

12.11 USE OF INFORMATION AND PUBLICATION

All information, including but not limited to, information regarding investigational product or the Sponsor's operations (e.g. patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the Sponsor to the Investigator and not previously published, and any data generated as a result of this study are considered confidential and remain the sole property of the Sponsor. The Investigator agrees to maintain this information in confidence, to use this information only to accomplish this study and not to use it for other purposes without the Sponsor's prior written consent.

The Investigator understands that the information generated in this clinical study will be used by the Sponsor in connection with the continued development of the IMP, and thus may be disclosed as required to other clinical Investigators or regulatory agencies. To permit information derived from the clinical studies to be used, the Investigator is obliged to provide the Sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated under the responsibility of the Sponsor and will contain eSource system data from all clinical units that participated in the study.

Clinical narratives may be written for the following events (for example):

- All deaths (irrespective of drug relationship);
- All other SAEs and AESIs during treatment with the IMP;
- All discontinuations of the IMP due to AEs (irrespective of drug relationship);
- At the discretion of the team and after statistical analysis of the data, certain discontinuations not related to AEs or treatment failure, i.e. related to lost to follow-up or withdrawal of consent (irrespective of treatment group);
- Any events of special interest explicitly requested by the regulatory agencies.

The PI will sign off the final version of the Clinical Study Report. A summary of this final version will be provided to the Investigators, the applicable regulatory authorities and the IECs/IRBs, if required by the applicable regulatory requirements, within 1 year after the end of the study (LPLV).

The Sponsor shall have the right to publish study data and information without approval from the Investigator. If an Investigator wishes to publish information from the study, a copy of the manuscript must be provided to the Sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations or other materials. If requested by the Sponsor in writing, the Investigator will withhold such publication for up to an additional 60 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or regulatory compliance, the Sponsor will review these issues with the Investigator. The Sponsor will not mandate modifications to scientific content and does not have the right to suppress information. For multicentre study designs and substudy approaches, results may need to be published in a given sequence (e.g. substudies should generally not be published before the primary endpoints of a study have been published). Similarly, Investigators will recognize the integrity of a multicentre study by not publishing data derived from an individual clinical unit until the combined results from the completed study have been published in full, within 12 months after conclusion, abandonment or termination of the study at all clinical units, or the Sponsor confirms there will be no multicentre study publication. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

12.12 REGISTRATION OF CLINICAL STUDIES AND DISCLOSURE OF RESULTS

Public disclosure of the study is under the responsibility of the Sponsor. The study will be registered on the ClinicalTrials gov site.

12.13 CONFIDENTIALITY

All study documents are provided by the Sponsor to the Investigator and appointed clinical staff in confidence. None of this material may be disclosed to any party not directly involved in the study without the Sponsor's written permission.

The Investigator must assure that subjects' anonymity will be maintained. The Investigator will keep a separate list with at least the initials, the subjects' study numbers, names, addresses and telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from the Sponsor.

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ATTACHMENT 1: EXAMPLE OF CLINICAL SCORE FOR MALARIA

Grading of the signs and symptoms specified in the table beneath will be performed at all relevant protocol-specified time points over the past 24 hours in accordance with the following:

- Absent = 0
- Mild = 1
- Moderate = 2
- Severe = 3

Subject ID:	**Date:**	**Time of Onset:** **Stop:**
Symptom/Sign	**Score (0 to 3)**	**qPCR**
Headache		☐ positive ☐ negative
Myalgia (muscle ache)		
Arthralgia (joint ache)		
Fatigue/lethargy		
Malaise (general discomfort/uneasiness)		
Chills/Shivering/Rigors		
Sweating/hot spells		
Anorexia		
Nausea		
Vomiting		
Abdominal discomfort		
Fever		
Tachycardia		
Hypotension		

ATTACHMENT 2: BECK DEPRESSION INVENTORY

Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
- 0 I do not feel sad.
- 1 I feel sad
- 2 I am sad all the time and I can't snap out of it.
- 3 I am so sad and unhappy that I can't stand it.

2.
- 0 I am not particularly discouraged about the future.
- 1 I feel discouraged about the future.
- 2 I feel I have nothing to look forward to.
- 3 I feel the future is hopeless and that things cannot improve.

3.
- 0 I do not feel like a failure.
- 1 I feel I have failed more than the average person.
- 2 As I look back on my life, all I can see is a lot of failures.
- 3 I feel I am a complete failure as a person.

4.
- 0 I get as much satisfaction out of things as I used to.
- 1 I don't enjoy things the way I used to.
- 2 I don't get real satisfaction out of anything anymore.
- 3 I am dissatisfied or bored with everything.

5.
- 0 I don't feel particularly guilty
- 1 I feel guilty a good part of the time.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6.
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7.
- 0 I don't feel disappointed in myself.
- 1 I am disappointed in myself.
- 2 I am disgusted with myself.
- 3 I hate myself.

8.
- 0 I don't feel I am any worse than anybody else.
- 1 I am critical of myself for my weaknesses or mistakes.
- 2 I blame myself all the time for my faults.
- 3 I blame myself for everything bad that happens.

9.
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10.
- 0 I don't cry any more than usual.
- 1 I cry more now than I used to.
- 2 I cry all the time now.
- 3 I used to be able to cry, but now I can't cry even though I want to.

11.

0	I am no more irritated by things than I ever was.
1	I am slightly more irritated now than usual.
2	I am quite annoyed or irritated a good deal of the time.
3	I feel irritated all the time.

12.

0	I have not lost interest in other people.
1	I am less interested in other people than I used to be.
2	I have lost most of my interest in other people.
3	I have lost all of my interest in other people.

13.

0	I make decisions about as well as I ever could.
1	I put off making decisions more than I used to.
2	I have greater difficulty in making decisions more than I used to.
3	I can't make decisions at all anymore.

14.

0	I don't feel that I look any worse than I used to.
1	I am worried that I am looking old or unattractive.
2	I feel there are permanent changes in my appearance that make me look unattractive
3	I believe that I look ugly.

15.

0	I can work about as well as before.
1	It takes an extra effort to get started at doing something.
2	I have to push myself very hard to do anything.
3	I can't do any work at all.

16.

0	I can sleep as well as usual.
1	I don't sleep as well as I used to.
2	I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3	I wake up several hours earlier than I used to and cannot get back to sleep.

17.

0	I don't get more tired than usual.
1	I get tired more easily than I used to.
2	I get tired from doing almost anything.
3	I am too tired to do anything.

18.

0	My appetite is no worse than usual.
1	My appetite is not as good as it used to be.
2	My appetite is much worse now.
3	I have no appetite at all anymore.

19.

0	I haven't lost much weight, if any, lately.
1	I have lost more than five pounds.
2	I have lost more than ten pounds.
3	I have lost more than fifteen pounds.

20.

0 I am no more worried about my health than usual.
1 I am worried about physical problems like aches, pains, upset stomach, or constipation.
2 I am very worried about physical problems and it's hard to think of much else.
3 I am so worried about my physical problems that I cannot think of anything else.

21.

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I have almost no interest in sex.
3 I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY

Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below.

Total Score	Levels of Depression
1-10	These ups and downs are considered normal
11-16	Mild mood disturbance
17-20	Borderline clinical depression
21-30	Moderate depression
31-40	Severe depression
over 40	Extreme depression

ATTACHMENT 3: NORMAL RANGES FOR VITAL SIGNS AND ECG

NORMAL RANGES FOR VITAL SIGNS

Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Pulse rate (bpm)	Oral temperature (°C)
$90 \leq SBP \leq 150$	$45 \leq DBP \leq 90$	$40 \leq pulse \leq 100$	$35.0 \leq t° \leq 37.5$

These normal ranges are applicable in supine and sitting position (after 5 minutes) and, only for Cohort 1, in the standing position (after 2 minutes).

NORMAL RANGES FOR ECG PARAMETERS

PR (ms)	QRS (ms)	QTc F (ms)	Heart rate (bpm)
$120 \leq PR \leq 220$	$QRS \leq 120$	$QTc \leq 450$	$40 \leq HR \leq 100$

These normal ranges are applicable in supine and sitting position (after 10 minutes).