CLINICAL STUDY PROTOCOL

Sponsor's Reference Number: MMV_P218_15_01

Richmond Pharmacology Study Number: C16009

EudraCT Number: 2016-001933-29

TITLE:	A Phase I study to investigate the safety, tolerability and pharmacokinetic profile and food effect of P218 in healthy adult volunteers
PHASE:	Phase 1
DRUG:	P218
SPONSOR:	Medicines for Malaria Venture
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Protocol Version and Date:	Version 4.0 31 Oct 2017

1. PROTOCOL APPROVAL SIGNATURES

Version 4.0 dated 31 Oct 2017

Sponsor's Approval

This protocol has been approved by MMV.

Sponsor's Signatory:

Name: **Dr Emilie Rossignol**

Signature: _____ **Date:** _____

Name: **Dr Stephan Chalon**

Signature: _____ **Date:** _____

Name: **Dr Stephan Duparc**

Signature: _____ **Date:** _____

1.1 Investigator's Agreement

I have read this MMV Protocol No. MMV_P218_15_01:

A Phase I study to investigate the safety, tolerability and pharmacokinetic profile and food effect of P218 in healthy adult volunteers

I have fully discussed the objectives of this trial and the contents of this protocol with MMV representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical/regulatory review of the trial, without written authorisation from MMV. It is, however, permissible to provide information to a subject in order to obtain consent.

I agree to conduct this trial according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with ICH guidelines on GCP and with the applicable regulatory requirements.

I understand that MMV may decide to suspend or prematurely terminate the trial at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial I will communicate my intention immediately in writing to MMV.

Principal Investigator:

Dr Ulrike Lorch, MD FRCA FFPM

Signature: U. Lorch **Date:** 8 NOV 2017

2. STUDY PERSONNEL

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3. TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

4. LIST OF ABBREVIATIONS

Abbreviation	Explanation
ACE	: Angiotensin-converting enzyme
ACT	: Artemisinin containing combination therapies
AESI	: Adverse event of special interest
ALT	: Alanine aminotransferase
API	: Active pharmaceutical ingredient
AUC_{0-t}	: The area under the plasma (or serum or blood) concentration-time curve from time zero to time 't' where t is a defined time point after administration
AUC_{inf}	: The area under the plasma (or serum or blood) concentration-time curve from time zero to infinity
AUC_{last}	: The area under the plasma (or serum or blood) concentration-time curve from time zero to the time of the last quantifiable concentration
BZD	: Benzodiazepine
Ca	: Calcium
CEREP	: A broad screen of receptors, enzymes and channels
CL	: Clearance
CL/F	: Apparent total clearance of the drug from plasma after oral administration
C_{max}	: The observed maximum plasma (or serum or blood) concentration following drug administration
CNS	: Central nervous system
CRF	: Case report form
CYP	: Cytochrome P450
DBP	: Diastolic blood pressure
DNA	: Deoxyribonucleic acid
DHFR	: Dihydrofolate reductase
DRF	: Dose range finding
ECG	: Electrocardiogram
ED90	: Effective dose for 90% of the animals
F	: Oral bioavailability

FSH	: Follicule stimulating hormone
GABA	: Gamma-aminobutyric acid
GCP	: Good clinical practice
GI	: Gastrointestinal
GLP	: Good Laboratory Practice
GMP	: Good Manufacturing Practice
G6PDH	: Glucose 6-phosphate dehydrogenase
Hb	: Hemoglobin
hERG	: Human Ether-à-go-go related gene
HEK	: Human embryonic kidney cells
HPMC	: Hydroxypropylmethylcellulose
HR	: Heart rate
huRBC	: Human red blood cells
IC90	: Median Inhibitory Concentration (concentration that reduces the effect by 90%)
ICF	: Informed consent form
ICH	: International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IPTp	: Intermittent preventive treatment during pregnancy
IV	:Intravenous
K	: Potassium
K_i	: Inhibtion constant
LC-MS/MS	: Liquid chromatography coupled to tandem mass spectrometry
LH	: Luteinizing hormone
LLOQ	: Lower limit of quantification
MAD	: Multiple ascending dose
MAO-B	: Monoamine oxidase B
M:E	: Myeloid: erythroid ratio
MIC	: Minimum Inhibitory Concentration (minimum concentration that will inhibit growth of the target pathogen)

MTD	: Maximum tolerated dose
Na	: Sodium
NADPH	: Nicotinamide adenine dinucleotide phosphate
NCE	: New chemical entity
NHMRC	: National statement on ethical conduct in human research
NOAEL	: No observed adverse effect level
OTC	: Over the counter
P_{app}	: Apparent permeability
PD	: Pharmacodynamic
Pgp	: P-glycoprotein
PK	: Pharmacokinetic
PO	: Oral dose
PRR	: Parasite reduction ratio
R_0	: Accumulation ratio
RH	: Relative humidity
RBC	: Red blood cell
SBP	: Systolic blood pressure
SAD	: Single ascending dose
SAE	: Serious adverse event
SCID	: Severe combined immunodeficiency
SD	: Single dose
SMFA	: Standard membrane feeding assay
SRT	: Safety review team
SUSAR	: Suspected, unexpected serious adverse reaction
TPMT	: Thiopurine S-methyl transferase
$t_{1/2}$	The terminal elimination half-life
T_{max}	The time to reach the maximum concentration after drug administration
UDPGA	: Uridine 5'-diphospho-glucuronic acid
UGT	: Uridine diphosphoglucuronosyl transferase

Vss	: Steady state volume of distribution
Vz/F	: Apparent volume of distribution during terminal phase after non-intravenous administration
WHO	: World Health Organization
WCBP	: Women of child bearing potential
WNCBP	: Women of non-childbearing potential

5. STUDY SYNOPSIS

Protocol Ref. MMV_P218_15_01	**Study drug:** P218

Title of the study: A Phase I study to investigate the safety, tolerability and pharmacokinetic profile and food effect of P218 in healthy adult volunteers

Principal Investigator:
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Study Parts: A, B	**Clinical phase:** 1 (The study does not have therapeutic or prophylactic intent and does not plan to assess efficacy)

Objectives:

Primary

- To investigate the safety and tolerability of single escalating oral doses of P218 when administered to healthy volunteers (men and WNCBP) under fasted conditions.

Secondary

- To describe the pharmacokinetics of P218 and its major metabolites (P218 β acyl glucuronide, P218-OH and P218-OH β acyl glucuronide) in healthy volunteers (men and WNCBP) after administration of single escalating oral doses
- To investigate the effect of a high fat meal on the pharmacokinetics and safety/tolerability of P218.

Exploratory

- To perform ex-vivo bioassay analysis against malaria parasites to confirm that P218 β acyl glucuronide is active against *Plasmodium* parasite and determine if other active metabolites are formed.
- To assess the effects of P218 on serum folate levels in healthy subjects
- To evaluate the cardiovascular safety profile of P218, assessing qualitative and quantitative ECG variations from baseline following dosing, in particular any effects on the QTc interval.
- To identify inherited genetic factors which may (1) predict response to treatment with P218, (2) explain variability in drug PK/PD, (3) predict susceptibility to drug-drug interactions, or (4) predict the occurrence of safety issues. The aim of such exploratory research will be to develop a better understanding of intrinsic and extrinsic factors that may affect the pharmacokinetics of P218 in human subjects.

Endpoints:

Primary

- Safety and tolerability of P218: Incidence, severity and relationship to the investigational product of observed and self-reported adverse events following administration of a single oral dose of P218 to healthy volunteers under fasted conditions.

Secondary

- Estimation of the following PK parameters following administration of a single dose of P218 (in fasted and fed cohorts) using non-compartmental methods: AUC_{last}, AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$,MRT, CL/F (for parent only), V_z/F (for parent only) and metabolites ratio.

Exploratory

- To determine the efficacy of P218 against parasites using an ex-vivo malaria assay : IC_{50}
- Absolute change from baseline P218 on serum folate levels over time
- The paired PK and QTc interval parameters pre-dose compared to post-administration of P218. Clinically significant ECG morphology and interval changes from baseline.
- Exploration of CYP and Uridine diphosphate glucuronosyltransferase (UGT) isoforms. |Exploration of other enzymes and transporters in accordance with evolving data.

Study Design:

The study is divided into two parts:

Part A

This is a double-blind randomised, placebo-controlled, parallel group, ascending dose study and will comprise seven fasted cohorts (8 volunteers in each) that will receive a single, ascending dose (SAD) of P218 to assess its safety, tolerability and pharmacokinetic profile. Each subject will participate in only one dose group and will receive only one dose of study drug. In each cohort, 2 and 6 subjects will be randomized to placebo and P218, respectively. The data obtained from each cohort will undergo a formal review by the Safety Review Team (SRT). SRT will confirm that it is safe to proceed with the next dose/cohort.

Part B

This is the pilot food effect evaluation. The dose is to be selected so that the predicted P218 exposure remains 3 fold below the maximum observed exposure achieved in part A and considered to be safe and well tolerated (in order to account for a possible increase in exposure with food). A new cohort of 8 subjects (all receiving active drug) will be evaluated for food effect in an open-label, randomized fed/fasted crossover design. Subjects participating in this food effect cohort will be randomized to two single dose sessions (fed/fasted). The second dose will be administered after a washout period of at least 5x observed human T1/2, to be confirmed once PK data are available from the relevant doses from Part A.

Diagnosis and main criteria for admission:

Healthy male and/or female (of non-child bearing potential) subjects will be included if they are aged between 18 and 45 years with a body mass index (BMI) between 18-25 kg/m^2; and a total body weight >50 kg (110 lbs), male subjects must agree to use an effective contraceptive method.

Main exclusion criteria are: male subjects with a female partner(s) who is (are) pregnant or lactating from the time of the administration of study medication, women of childbearing potential (WCBP), subject has any surgical or medical condition possibly affecting drug absorption (e.g. cholecystectomy, gastrectomy, bowel disease, etc.), distribution, metabolism or excretion. Subjects with a medical history of cholecystitis and/or cholelithiasis. Subject has a clinically significant disease, allergy or clinical significant abnormal laboratory, ECG, vital signs or other safety findings as determined by medical history, physical examination or other evaluations conducted at screening or on admission. Subjects with documented megaloblastic anaemia secondary to folate deficiency. Subject has Serum folate levels below normal range (as determined by the local laboratory reference ranges) at screening.

Test treatment(s) and mode of administration:

Part A (Anticipated doses)

Cohort 1: 10mg (one oral single dose of 10 mg)

Cohort 2: 30mg (three oral single doses of 10 mg)

Cohort 3: 100mg (two oral single doses of 50 mg)

Cohort 4: 250mg (one oral single doses of 250 mg)

Cohort 5: 500mg (two oral single doses of 250 mg)

Cohort 6: 750mg (three oral single doses of 250 mg)
Cohort 7: 1000mg (four oral single doses of 250 mg)

Anticipated doses will be determined after satisfactory review of all safety, tolerability and PK data by the Safety Review Committee in accordance with the Adaptive Features (

Table 1), rules for dose escalation/progression and toxicity.

Part B
Anticipated doses will be determined after satisfactory review of all safety, tolerability and PK data by the Safety Review Committee in accordance with the Adaptive Features (

Table 1), rules for dose escalation/progression and toxicity.

Reference treatment(s) and mode of administration:
Oral dose of placebo to match P218.

Anticipated duration of treatment:
Part A
A single oral dose of P218 per study cohort on Day 1.

Part B
A single oral dose of P218 under fasted or fed conditions in two different dosing periods.

Criteria for evaluation:

Safety

Safety and tolerability will be evaluated at regular time-points up to the last follow-up visit for reported adverse events (AEs), scheduled physical examinations, vital sign measurements (including orthostatic changes in BP and HR), cardiac rhythm monitoring (telemetry), 12-lead triplicate ECGs, and clinical laboratory test results.

Pharmacokinetic

Blood and urine samples will be obtained to determine the PK of orally administered P218.
Part A and Part B
Venous blood samples (0.5mL) will be obtained within 2 hours before P218 administration and at 0.25, 0.5, 1, 2, 4, 6, 7, 8, 12, 24, 48, 72, 120, 168, 192 and 240 h after administration (Part A). For those subjects participating in the food-effect group (Part B), blood samples will be collected at the same times during both periods (fed/fasted). These time points will be; 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72 and 120 h after administration.
Single-dose PK values such as C_{max}, $T_{1/2}$ and AUC (parent and metabolites) will be derived from the plasma concentration versus time and urinary excretion data.
Urine samples will be collected for determination of P218 concentrations on Day 1 before test article administration and at 0 to 6, 6 to 12, 12 to 24, and 24 to 48 hours after administration (Part A only). The date, actual collection time, pH, collection volumes, and urine creatinine will be recorded in the source documents. For subjects participating in the food-effect group, urine samples will not be collected.
A non-compartmental PK method, as appropriate, will be used to analyze the plasma concentrations of P218, P218 β acyl glucuronide, P218-OH and P218-OH β acyl glucuronide and urine concentrations of P218.
Urine PK analysis for P218 will be conducted with the highest dose cohort achieved in the study [1 cohort] initially, using a non-validated assay. If significant amounts are detected [greater than 10% is detected], urine from this cohort and the lower dose cohorts will be assayed with a validated assay. If minimal amounts are detected [less than 10% is detected] in this cohort, no further analysis will be conducted (to be confirmed).

Pharmacodynamics

- **Malaria ex-vivo assay**

For Malaria ex-vivo assay, samples of approximately 2.5 mL of whole blood will be collected at the same time as PK samples into a serum separating tube (SST).

These samples will be used for future bioassay analysis against malaria parasites *in vitro* to confirm that P218 β acyl glucuronide metabolite is active against *Plasmodium* parasite. The samples will be stored frozen at the clinical site at -15 °C or lower pending analysis.

- **Serum and Red Blood Cell Folate**

Part A:

Samples of venous blood will be collected at the same time as safety samples (at screening, on admission and 24, 72 and 240 hours after administration of P218).

Part B:

Samples of venous blood will be collected at the same time as safety samples (at screening, on admission and 24, 72 and 120 hours after administration of P218).

These samples may be used for potential future analysis of the possible effect of P218 on folate levels.

- **Intensive cardiac assessments (Part A only)**

Triplicate 12-lead ECGs will be taken in parallel with every PK sample, enabling a potential PK/PD analysis of QT/QTC changes compared to baseline (3x triplicate ECGs pre-dose). In addition, 3 triplicate ECGs will be taken within 4 hours post the scheduled lunch to confirm validity of the 12-lead ECGs by demonstrating shortening of the QTc interval.

Statistical Methods:

A statistical analysis plan (SAP) containing detailed statistical methodology will be written and signed off before the unblinding of the study. The plan will be updated to reflect Adaptive Features of the study as appropriate.

Statistical analysis of safety parameters

All subjects that were enrolled and received a dose of the study drug will be included in the data analysis. In Part A, subjects will be analysed as treated (i.e. in accordance with the study drug received, even if different from the one to which they were randomised). In all treatment and dose group comparisons for Part A, data from all subjects who received placebo will be pooled.

The subject disposition will be summarised. Data for background and demographic variables will be listed and summarised descriptively by treatment and dose. Medical history, current medical conditions, results of laboratory screening tests, drug tests and any other relevant baseline information will be listed by treatment, dose and subject. Previous and concomitant medication will be listed per treatment and dose level.

Adverse event data will be summarised by treatment, dose, MedDRA system organ class, MedDRA preferred term and severity (WHO grading). Vital signs, routine safety laboratory data and ECG parameters will be summarised descriptively by treatment, dose, and time point. Absolute values and change from baseline will be presented. A categorical analysis of QTcF changes from baseline will be presented. Listings of clinically relevant abnormal laboratory results will be generated.

Statistical analyses of pharmacokinetic parameters

The following pharmacokinetic parameters will be determined using non-compartmental method(s) from plasma concentration-time data from all dose cohorts: AUC_{last}, AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$, MRT, CL/F (for parent only), V_z/F (for parent only) and metabolites ratio. These data will be summarized descriptively by treatment and dose.

Statistical analyses of exploratory data

Pharmacodynamic parameters

- **Serum folate levels**

Serum Folate Levels values and changes from baseline will be listed and summarised using descriptive statistics per treatment and time point including graphs as applicable.

- **Ex-vivo malaria assay**

Analysis of bioassay sample results may be performed to assess the efficacy of the P218.

- **ECG analysis**

ECG analysis will be compliant with the correct recording and manual adjudication of ECGs in thorough QT/QTc studies.

Pharmacogenetics

Pharmacogenetics samples will be stored for future optional analysis.

Rationale for sample size

Part A

Because the primary objective is an initial assessment of safety, each treatment group is limited to 6 subjects receiving P218. Administration of P218 to 6 subjects in each dose group provides a 47%, 62%, 74%, or 82% probability of observing at least 1 occurrence of any AE with a true incidence rate for a given dose group of 10%, 15%, 20%, or 25%, respectively.

Furthermore, it is assumed that pooling the data for the 2 placebo subjects who received placebo from each cohort will provide an adequately sized control group.

Part B

This is a pilot evaluation designed to determine whether P218 PK is impacted by food. Historically 8 participants in a dose cohort have proven sufficient to characterize the preliminary effects of food on safety and PK of a new chemical entity in healthy subjects. Therefore, the size of the current cohort (n=8) is considered adequate at this stage of the drug development process.

6. INTRODUCTION

Malaria is an infectious disease that threatens half of the world's population and is caused mainly by two protozoan parasites: *Plasmodium falciparum* and *Plasmodium vivax*. The World Health Organization (WHO) reported 198 million cases of the disease in 2013 out of an estimated 3.2 billion people at risk for the disease. In the same year, malaria caused 584,000 deaths; 90% of the mortality was in Africa, with children <5 years of age attributing 78% of deaths (World Malaria Report, 2014). 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 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 due to increasing resistance of the current antimalarial therapies, new antimalarial therapies are required to partner with existing therapies to address a growing unmet medical need.

P218 was discovered by a consortium consisting of MMV, the London School of Hygiene and Tropical Medicine, Monash Centre for Drug Candidate Optimisation and the National Center for Genetic Engineering and Biotechnology (BIOTEC), a Thai research organization, with whom MMV is currently collaborating.

P218 is a selective inhibitor of *Plasmodium* DHFR, an enzyme which catalyses the reduction of folates to tetrahydrofolates, which are essential for DNA biosynthesis in the malarial parasite (Yuthavong et al, 2012). DHFR inhibitors, which are selective to the *Plasmodium* DHFR vs the 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. However, its usage for malaria treatment was stopped in many areas following emergence of resistance. 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 is highly active on *P. yoelli*, *P. chabaudi* and *P. berghei* liver stage parasites, which are *Plasmodium* strains infecting murine species. These *Plasmodium* strains are used as murine models to evaluate compound potential activity on *P. falciparum* and *P. vivax* infecting the human liver. Recently, P218 was also shown to be active on *P. falciparum* liver stage. Targeting human liver parasites is essential for a compound to provide chemo protection against malaria. Pyrimethamine is also active on these *Plasmodium* parasites, albeit less than P218, and is now a component of the standard of care as second and third trimester Intermittent Preventive Treatment during Pregnancy (IPTp) in high malaria transmission areas.

Due to its profile combining activity on pyrimethamine resistant strains and potential action on liver stage parasites, P218 is primarily considered for malaria chemo-protection. Acute uncomplicated malaria is also under consideration as a second indication for the new chemical entity (NCE).

There have not been any concerns relating to safety, tolerability or PK data in 48 subjects in the 6 first cohorts tested up to 750mg in the FIH study. However, preliminary PK investigation revealed the presence of two β acyl glucuronides metabolites formed in large proportion in human upon oral administration of P218.

The potential reactivity of these acyl glucuronides is considered to be due to the chemical stability and rearrangement of the conjugate to interact with proteins. Given that the structure and in particular the site of conjugation is identical between both acyl glucuronides of P218, it is considered appropriate to assess safety in terms of total acyl glucuronide burden rather than consider each one independently.

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 AUClast being the sum of the human AUClast 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 (refer to the pharmacokinetic properties paragraph below).

Thus, it is considered safe to continue the FIH study and perform a 1000 mg dose cohort as originally planned.

6.1 Pharmacokinetic properties

Non-Clinical Studies

P218 and its major glucuronide metabolite (P218 β glucuronide) single-dose PK values were derived from the plasma concentration versus time and urinary excretion data obtained in animals.

Plasma protein binding of P218 was moderate in all species, with no evidence of concentration dependency across the concentration range assessed.

In animal species, following IV administration, P218 has moderate to high plasma clearance (~50% liver blood flow across species) and medium, consistent steady state volume of distribution in all species resulting in moderate terminal half-life ($T_{1/2}$) values of several hours from mouse to dog. The predicted $T_{1/2}$ is comprised in the range [31h–40h].

Following single oral administration, P218 is rapidly absorbed in all species tested showing acceptable oral bioavailability of ~20-50% with enterohepatic recirculation observed in some animals. In a study in rats, the brain to plasma concentration ratio was very low suggesting that there was minimal distribution of P218 into the brain.

Following repeated dose administration of P218 to rats and dogs for 14 days, P218 and P218 β glucuronide generally increased in a dose proportional manner with respect to dose. In rats as well as in dogs no gender difference was observed for P218 at steady state (Day 14). No evidence of accumulation of parent or P218 β glucuronide metabolite was observed.

Metabolites with primary (direct) glucuronidation (the P218 β glucuronide) and monooxygenation have been identified. Essentially all of the P218 dose in a bile duct cannulated rat study was excreted in bile, mostly as P218-glucuronide suggesting that glucuronidation followed by biliary excretion may represent the major route of elimination. In a different rat PK study, P218 was also eliminated via the urine with approximately 18% of the

dose being recovered in rat urine suggesting that renal clearance may constitute a second independent clearance pathway (however apparently smaller). It is unlikely that oxidative Phase 1 metabolism constitutes a major clearance mechanism of P218 since mono-oxygenation was shown to be a relatively minor metabolic pathway for P218. With and without pre-incubation, P218 did not cause significant inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 suggesting little or no potential to produce drug-drug interactions via CYP450´s pathways.

A quantitative analysis of human plasma from the FIH study has been conducted using LC MS/MS. According to the preliminary data collected, three major metabolites were identified in vivo by comparison to reference standards: P218 β acyl glucuronide conjugate, P218 OH and P218 OH β acyl glucuronide conjugate. These 3 metabolites are identical to the ones detected in the single dose rat and dog PK studies.

To evaluate exposure to P218-OH and P218-OH β acyl glucuronide, additional studies in the rat and the dog were performed where these metabolites were quantified following a single dose of P218. Single doses of 100 and 250 mg/kg to rats and a single dose of 20 mg/kg to dogs were administered and plasma concentrations measured. These doses were the highest tolerated doses in the 14 day (or 7 day) toxicology studies. In rats, calculation of the proportion of circulating P218 β acyl glucuronide as a ratio to the total circulating drug related material (∑AUClast of P218, P218-OH, P218 β acyl glucuronide and P218-OH P218 β acyl glucuronide) resulted in values of 18 to 22%. In the dog, this ratio was 43%. Applying the same calculation method, ratios of P218-OH and P218-OH β acyl glucuronide were <1% in both species.

Clinical Studies

A quantitative analysis of human plasma from this ongoing FIH study has been conducted using LC MS/MS. According to the preliminary data collected, three major metabolites were identified in vivo by comparison to reference standards: P218 β acyl glucuronide conjugate, P218 OH and P218 OH β acyl glucuronide conjugate. These 3 metabolites are identical to the ones detected in the single dose rat and dog PK studies

Calculation of the proportion of circulating P218-OH as a ratio to the total of circulating drug related material in plasma (AUClast of P218, P218 β acyl glucuronide, P218-OH and P218 β acyl glucuronide) results in values below 10% in human. With reference to ICH M3 and the FDA guidance for Safety Testing of Drug Metabolites (Metabolites in Safety Testing, MIST guidance) this metabolite is therefore considered to be qualified.P218 β acyl glucuronides (with and without the hydroxyl group on the phenyl ring) were prominent metabolites in healthy volunteers' plasma, accounting for 72-78% of total drug related material.

Individually these acyl glucuronides exist at circulating concentrations higher than 10%, however, the total acyl glucuronide exposure in the animal studies (rats and dogs: 37.2 to 74.6 µg•h/mL) are similar to, or greater than, the total acyl glucuronide exposure in humans (48.2 µg•h/mL).

Given that the acyl glucuronide structure, potentially responsible for protein acylation, is identical in each metabolite, the chemical reactivity is considered to be the same and therefore, like the P218-OH metabolite, they are considered qualified according to MIST and ICH M3 (see the IB, sections 5.2.4 and 5.3.9.1, for more information).

Additionally, preliminary PK data from FIH cohorts 1-6 (up to single doses of 750mg) has shown that at 72hrs post dose, negligible amounts of P218 and its main metabolites (P218 OH, P218 β acyl glucuronide and P218 OH β acyl glucuronide) remain in circulation. It is therefore very unlikely that any biological activity will be observed beyond this timepoint.

6.2 Rationale for conducting study

The main purpose of this phase 1 study with this selective Plasmodium DHFR inhibitor P218 is to generate safety, tolerability and pharmacokinetic (PK) data when first administered to healthy men and women of non-childbearing potential (WNCBP) as escalating single ascending doses (Part A) under fasted conditions. In addition, the potential effect of food consumption on the previously stated objectives will be assessed. Furthermore, the study shall assess the effects of P218 against Plasmodium parasite and on serum folate levels, and identify the genetic factors that may affect the PK of P218 in human subjects.

The rationale for taking P218 into further development is the expectation that this compound could be effective for the treatment of pyrimethamine P. falciparum resistant strains. Prior to initial testing in patients, the safety and the pharmacokinetic characteristics of the drug in healthy subjects must be investigated.

6.3 Risk-benefit evaluation

6.3.1 Potential Benefits

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

6.3.2 Potential Risks

6.3.2.1 Non-clinical studies

The toxicology testing of P218 in animals and in vitro has been conducted to support oral dosing in humans in accordance with ICH guidelines (ICH M3 [R2]). The pivotal toxicology, genetic toxicology and safety pharmacology studies were conducted in accordance with GLP and currently accepted guidance with respect to duration, animal numbers and dose level selection. There are no known reasons to exclude the rat or dog as relevant for evaluating toxicity.

P218 was evaluated in a wide range of receptor binding assays at a concentration of 10 µM. No major inhibition of binding was observed except against thromboxane A2/PGH2. A functional assay confirmed antagonist activity with an IC_{50} of 19 µM. An in vitro assessment of platelet aggregation in rat blood showed P218 to have no effect on aggregation mediated via either the Arachidonic Acid or Collagen pathways at a concentration up to 53µM/21.1µg/mL (a

concentration selected to exceed both the IC_{50} and the maximum concentration observed in the rat 14 day toxicology study).

P218 plasma protein binding studies in rat, dog and humans showed small differences in free fraction with the free fraction in humans (6.3%) lower than in rats (11.4%) or dogs (21.5%). These differences were taken into account in assessment of toxicity and dose response.

P218 was assessed in a series of exploratory and definitive repeat dose oral toxicity studies up to 14 days in duration in rats and dogs, genetic toxicology studies for mutagenicity and clastogenicity and phototoxicity studies. The oral route of exposure was selected for these studies since it is the intended route of clinical exposure.

P218 was well tolerated following single dose administration. In the rat doses up to 2000 mg/kg P218 resulted in no mortality, no important clinical signs and no post mortem changes. In the dog single doses up to 100 mg/kg were well tolerated with no clinical observations.

Repeated dose toxicity studies in both rat and dog demonstrated the gastrointestinal (GI) tract to be the main target for toxicity associated with P218. The primary mechanism of anti plasmodial activity is mediated through DHFR, an enzyme which catalyses the reduction of folates to tetrahydrofolates. Depletion of tetrahydrofolates, such as following dosing with methotrexate, has been associated with GI toxicity. At the highest doses administered in the pivotal 14 day studies (250 mg/kg/day in rat [UNX0029] and 40 mg/kg/day in dog [UNX0031]), P218 was not tolerated. At these doses, deterioration in the clinical condition of the animals necessitated the early termination of these groups. In a specific study to demonstrate GI toxicity was associated with depletion of tetrahydrofolates, P218 was administered to rats at 250 mg/kg/day with and without concomitant administration of folinic acid (UNX0052). A single 250 mg/kg dose of P218 did not induce any microscopic changes in the GI tract. Repeated dosing of P218 at 250 mg/kg/day induced GI pathology that was completely prevented when folinic acid was co administered. The IC_{50} of P218 against plasmodial DHFR (3.55 ng/mL) was substantially lower than the one needed to inhibit human DHFR (IC_{50} 460 ng/mL) giving confidence that efficacy could theoretically be achieved in malaria patients without significant inhibition of human DHFR and risk of GI adverse effects.

Although 10 mg/kg/day was found to be NOAEL dose level for in-life findings, the minor gall bladder changes identified in all males receiving 10 mg/kg/day prevented this dose being identified as a true NOAEL. The effects in the gall bladder showed evidence of reversibility and were consistent with the consequences of DHFR inhibition. 10 mg/kg/day can be considered as the dose associated with very minimal and reversible effect upon the gall bladder of the dog. As a NOAEL has not been identified, an additional safety factor of 3 was applied to calculate the study starting dose.

When tested in a five strain Ames test, P218 was not mutagenic in bacteria, with or without metabolic activation. Weak induction of micronuclei in V79 Chinese hamster cells using 24 hour exposure in the absence of metabolic activation was observed. Because of the weak activity in the in vitro cell assay, two in vivo tests were conducted. P218 was found to be negative in both a GLP rat bone marrow micronucleus test in the rat and in a GLP rat liver comet assay at doses up to 2000 mg/kg (maximum recommended according to ICH

guidelines). Since no evidence of activity was seen in the Ames test and two in vivo assays, it was concluded that P218 is not genotoxic.

A UV-visible spectrum was obtained for P218 and absorption was noted at 290 nm. Consequently, the compound was tested in a reactive oxygen species assay and was found to be inactive. It was concluded that P218 presents no phototoxic risk.

In vivo in NOD SCID mice engrafted with human A G6PDH deficient red cells, P218 showed no evidence of haemolytic toxicity as evidenced by comparable levels of human red blood cells (huRBC) to those found in A-huRBC NOD/ severe combined immunodeficiency (SCID) mice treated with vehicle alone.

The nonclinical safety profile of this compound has been adequately characterised to support progression into clinical trials: P218 was tested in a GLP hERG patch clamp assay and had no effect on hERG tail current at 100 μM; a concentration substantially higher than expected therapeutic maximal concentrations. No effects were seen in studies designed to evaluate the respiratory and nervous systems of rats following single doses up to 250 mg/kg. P218 had no effect on arterial blood pressure, heart rate, body temperature or cardiac conduction times in dogs given single doses up to 50 mg/kg that resulted in peak plasma concentrations up to 4740 ng/mL. An effect on QT prolongation and risk of Torsades de Pointes in humans is therefore considered to be low and was supported by absence of any effect in vivo in either telemetry studies or repeated dose toxicity assessment of electrocardiograms (ECGs).

Reproductive toxicity studies have been completed in rat and rabbit. These studies indicate P218 induces maternal toxicity in both rat (80 mg/kg/day) and rabbit (50 mg/kg/day). In the rat, doses not well tolerated by the dams (80 mg/kg/day) resulted in foetal abnormalities consistent with folate inhibition. In the rabbit, there were no P218 related minor or variant external, visceral or skeletal foetal abnormalities at the maximum dose that could be tested (30 mg/kg/day) due to maternal toxicity seen at 50 mg/kg/day and above. The NOAEL for maternal and embryo foetal toxicity in the definitive rat and rabbit studies was considered to be ≥32 and ≥30 mg/kg/day P218, respectively with corresponding exposures of 6240 and 34,900 ng•h/mL. Examination of both male and female reproductive organs in the repeated dose studies did not reveal any effects therefore supporting administration of P218 to males and women who do not have potential for pregnancy. Three major human metabolites were identified in vivo by comparison to reference standards: P218 β acyl glucuronide conjugate, P218 OH and P218 OH β acyl glucuronide conjugate. Metabolites ratio were calculated based on the Metabolites in Safety Testing (MIST) guidelines method by comparing a metabolite AUC to the sum of the AUC values of the total circulating drug related material.

P218-OH ratio to total drug related material was calculated to be less than 10%. With reference to ICH M3 and the FDA guidance for Safety Testing of Drug Metabolites (Metabolites in Safety Testing, MIST guidance) this metabolite is therefore considered to be qualified.

P218 β acyl glucuronides, with and without the hydroxyl group on the phenyl ring, were formed in large proportions in healthy volunteers accounting for 72-78% of total drug related material. The estimated P218 β acyl glucuronides global human AUC at a 1000 mg dose (48.2μg•h/mL) is expected to remain below the highest tolerated, estimated cumulative glucuronides AUCs in

the dog 14 day toxicology study (74.6 μg•h/mL) and the rat folate supplementation study (62.5 μg•h/mL).

In conclusion, 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. The potential reactivity of these acyl glucuronides is considered due to the chemical stability and rearrangement of the conjugate to interact with proteins. Given that the structure and in particular the site of conjugation is identical between both acyl glucuronides of P218, it is considered appropriate to assess safety in terms of total acyl glucuronide burden rather than consider each one independently.

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_{last} being the sum of the human AUC_{last} of each molecule. Under this assessment all metabolites are considered qualified as exposure in the toxicology species was similar to or 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.

6.3.2.2 Clinical Studies

To date, there are currently no completed clinical studies with P218, however in this ongoing study (MMV_P218_15_01) there have not been any concerns relating to safety, tolerability or PK data in 48 subjects in the 6 first cohorts testing up to 750mg. There are licensed anti-malarial treatments with the same mechanism of action, e.g. pyrimethamine.

Potential risks will be closely monitored for as part of the safety evaluations being performed in this study. Measures to minimise the risks to volunteers will include:

- Selection of a starting dose below a dose level for which effects are expected. Please refer to protocol section 8.4.1.
- Sentinel dosing of subjects in Part A (Cohorts 1, 2 & 3). Please refer to protocol section 8.4.5.
- Progression to consecutive study parts or higher exposure dosing regimens only after evaluation of data from previous parts/cohorts and approval from Safety Review Committee (SRC). Please refer to protocol section 8.2.3 and 8.2.4.12
- Clinical and laboratory monitoring of study subjects. Please refer to protocol section 12.
- 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.
- Predefined stopping rules based on safety and PK.

- Regular clinical chemistry (including Liver Function Tests and serum folate levels) and haematology blood tests will be performed at scheduled time points and if necessary will be repeated to ensure appropriate follow-up of any clinically relevant abnormality.

  Considering the risk management plan outlined in this protocol in conjunction with; adaptive boundaries, toxicity rules, the mode of action of the drug, the nature of the target, the relevance of the animal model, the toxicity profile and the fact that it is a single dose study, the overall risk to volunteers participating in the study is considered to be minimal and acceptable.

- The analysis of interim safety and tolerability data up to single doses of 750mg (SAD) indicated good safety and tolerability, and no ongoing safety concerns.

6.3.2.3 Serum folate levels

As mentioned in section 6.3.2.1 repeated dose toxicity studies in both rat and dog demonstrated the gastrointestinal (GI) tract to be the main target for toxicity associated with P218. A potential cause of this toxicity is thought to be depletion of tetrahydrofolates. It is considered that the risk of depletion of serum folate levels in humans is theoretical. Subjects will not be included if they have any gallbladder or longstanding gastrointestinal conditions. They will also be excluded if they have a history of megaloblastic anaemia secondary to folate deficiency. Subjects will have samples taken to measure their serum folate levels and (optional) red blood cell folate levels at specified time points during the study. These samples will be analysed based on clinical need.

7. STUDY OBJECTIVES AND OUTCOMES

7.1 Objectives

7.1.1 Primary objectives

- To investigate the safety and tolerability of single escalating oral doses of P218 when administered to healthy volunteers (men and WNCBP) under fasted conditions.

7.1.2 Secondary objectives

- To describe the pharmacokinetics of P218 and its major metabolites (P218-OH, P218 β acyl glucuronide and P218-OH β acyl glucuronide) in healthy volunteers (men and WNCBP) after administration of single escalating oral doses
- To investigate the effect of a high fat meal on the pharmacokinetics and safety/tolerability of P218

7.1.3 Exploratory objectives

- To perform ex vivo bioassay analysis against malaria parasites to confirm that P218 β acyl glucuronide is active against Plasmodium parasite and determine if other active metabolites are formed.

- To assess the effects of P218 on serum folate levels in healthy subjects.
- To evaluate the cardiovascular safety profile of P218, assessing qualitative ECG variations from baseline following dosing, in particular any effects on the QTc interval
- To identify inherited genetic factors which may (1) predict response to treatment with P218, (2) explain variability in drug PK/PD, (3) predict susceptibility to drug-drug interactions, or (4) predict the occurrence of safety issues. The aim of such exploratory research will be to develop a better understanding of intrinsic and extrinsic factors that may affect the pharmacokinetics of P218 in human subjects.

7.2 Endpoints

7.2.1 Primary endpoints

- Safety and tolerability of P218: Incidence, severity and relationship to the investigational product of observed and self-reported adverse events following administration of a single oral dose of P218 to healthy volunteers.

7.2.2 Secondary endpoints

- Estimation of the following PK parameters following administration of a single dose of P218 (in fasted and fed cohorts) using non-compartmental methods: AUC_{last}, AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$, MRT, CL/F (for parent only), V_z/F (for parent only) and metabolites ratio.

7.2.3 Exploratory endpoints

- To determine the efficacy of P218 against parasites using an *ex-vivo* malaria assay: IC_{50}.
- Absolute change from baseline P218 on serum folate levels over time
- The paired PK and QTc interval parameters pre-dose compared to post-administration of P218. Clinically significant ECG morphology and interval changes from baseline.
- Exploration of CYP and Uridine diphosphate glucuronosyltransferase (UGT) isoforms. Exploration of other enzymes and transporters in accordance with evolving data.

8. STUDY DESIGN

8.1 Overall study design and procedure

Part A

This is a randomized, double-blind, placebo-controlled, parallel group, Phase I study which will assess the safety, tolerability, pharmacokinetics of single (SAD) ascending oral doses of P218 in healthy subjects (men and WNCBP).

Part B

This is an open-label, randomised fed/fasted crossover study in healthy subjects (men and WNCBP) to examine the food effect of a high fat meal on the PK, safety and tolerability of P218.

This study incorporates the use of an adaptive design. Study specific adaptive features and their limits are described in

Table 1. Adaptive protocol features.

Table 1. Adaptive protocol features

Adaptive Study Design Areas	Features	Limits
Dose	1. All anticipated dosing levels can be adjusted in accordance with PK, safety and tolerability data collected up to the decision making time-point.	I. The PK derived mean exposure for a dosing regimen will not exceed the defined PK exposure limit (based on C_{max} and $AUC_{0\text{-}last}$). Please refer to section 8.3.3 **PART A:** II. The starting dose for Part A of the study will not exceed 10 mg. III. The highest dose for Part A of the study will not exceed 2000 mg. IV. The dose increments between the dose levels 1 to 5 in Part A will be no more than 4-fold. V. The dose increments between the next dose levels (from dose level 5 onwards) in Part A will be no more than 2-fold. **PART B:** VI. The planned dosing regimens' anticipated mean exposures will not exceed mean exposures (based on C_{max} and $AUC_{0\text{-}last}$) previously explored in Part A with acceptable safety and tolerability, i.e. exposure levels at which no study specific criteria stopping dose progression and/or escalation were met. VII. A potential food effect of no less than 3-fold will be taken into account when setting the dose for Part B.

Adaptive Study Design Areas	Features	Limits
Timing	2. Part B can overlap with Part A	I. Minimum data requirements for initiating each dose level in Parts A and B apply as outlined in Rules for escalation/progression.
	3. Cohorts/dosing regimens may be split into sub-groups.	I. A mandatory sentinel dosing strategy of dosing 2 subjects (1 subject on IMP, 1 subject on placebo) will be used for cohorts 1, 2 and 3 in Part A. If safety and tolerability is acceptable, the remaining subjects of this cohort can be dosed with a minimum interval of 24 hours following the dosing of the first subject of the sentinel group. Sentinel or sequential dosing for all cohorts and study parts can be performed at the discretion of sponsor and PI. II. If PK for cohorts 1, 2 and 3 are within expectation and safety tolerability is acceptable then no further sentinel dosing is necessary.
Flexible Cohort Sizes	4. Withdrawn subjects can be replaced at the discretion of the sponsor and PI. 5. Replacement subjects may be enrolled in an ongoing cohort, or dosed together as a group or dosed separately. 6. The number of subjects in a cohort in Part B can be extended to gather further information on this dose level.	I. The maximum extension of Part B is 100% of the original cohort size at the selected dosing regimen.
Samples and Assessments	7. The in-house stay may be shortened if: a. The evolving PK data demonstrates a shorter t½ than anticipated. b. The Safety Review Committee (SRC) considers it appropriate from a safety/tolerability point for an upcoming dose cohort. 8. The in-house stay or follow-up period may be prolonged if:	I. A minimum in-house period of 24 hours post dose. II. If the in-house stay is shortened the minimum in-house period for study cohorts will be based on evolving safety, tolerability, and PK data and will not be less than 2 x t½ of the IMP following the last dose.

Adaptive Study Design Areas	Features	Limits
	a. It is considered clinically necessary by the PI for individuals on a case-by-case basis. b. The Safety Review Committee (SRC) considers it necessary from a safety/tolerability point for an upcoming dose cohort. c. The follow-up period for a dose cohort may be prolonged if evolving PK data require a longer follow-up period.	I. A maximum extended in-house or follow-up period cannot be defined as the extension will be as long as necessary to ensure the safety of the individual participant(s). II. The maximum extended in-house or follow-up period for study cohorts will be based on evolving safety, tolerability, and PK data and will not usually exceed 10 x t½ of the IMP following the last dose.
	8. Additional safety blood and/or urine samples/variables may be taken or analyzed if: d. It is considered clinically necessary by the PI for individuals on a case-by-case basis. e. The SRC considers it necessary from a safety/tolerability point for an upcoming dose cohort.	I. For individuals, a maximum number of safety blood samples will be determined on a case-by-case basis and cannot be pre-defined as investigations will be performed as necessary to ensure the safety of the individual participants. II. Study specific maximum blood volume will not be exceeded (Please refer to section 12.12).
	9. Timing of blood and urine PK and/or exploratory assessments/PD may be adjusted in accordance with evolving data and dosing schedule. 10. Additional or less blood and urine PK/PD and/or exploratory assessments may be taken in accordance with evolving data and dosing schedule.	I. Minimum: sufficient PK samples to establish full protocol specific serum PK profile. II. Study specific maximum blood volume will not be exceeded (Please refer to section 12.12).
	11. Timing of safety assessments including but not limited to laboratory safety samples, vital signs and ECGs may be adjusted in accordance with evolving data and dosing schedule. 12. Additional safety assessments including but not limited to laboratory safety samples, vital signs and ECGs may be taken in accordance with evolving data and dosing regimens.	I. Alterations in timing of the safety assessments need to be a reflection of the established PK, safety and tolerability profile up to the decision making time-point. II. Alterations need to be made in the spirit of the current CSP (i.e. focus on the capture of essential and useful data) and not affect the risk profile of the study.
	13. Specialist referrals (e.g. to a cardiologist) may be made (and	I. A maximum for individuals will be determined on a case-by-case basis and

Adaptive Study Design Areas	Features	Limits
	may include all relevant assessments and investigations) if it is considered clinically necessary by the PI or Sponsor or SRC for individuals on a case-by-case basis.	cannot be pre-defined as investigations will be performed as necessary to ensure the safety of the individual participants.
	14. Except for those subjects that have been randomized, screening assessments, including Holter ECG recordings performed at Richmond Pharmacology Ltd on volunteers screened for another study can be used for this study to avoid unnecessary tests.	I. The assessments must meet protocol criteria (e.g. the method to be used). II. The assessments must be performed within the protocol defined screening window. III. Holter ECG recordings are valid for a period of three months.
	15. ECG analysis for the purpose of exploratory QT/QTc analysis / intensive cardiac assessments may be performed on selected or all dose levels in Part A. Under these circumstances : a. If PK sampling times are changed then the ECG sampling times will be changed accordingly, for the purpose of exploratory QT/QTc analysis/intensive cardiac assessments. **b.** If the meal times need to be changed, ECG times will also be changed in accordance to maintain the sampling schedule stipulated to capture the food effect, for the purpose of exploratory QT/QTc analysis/intensive cardiac assessments with confirmation of assay sensitivity. 16. Telemetry data gathered during the study may be used in the exploratory analysis of drug related QT/QTc interval changes.	I. For the purpose of exploratory QT/QTc analysis and to confirm assay sensitivity a minimum of three postprandial time points will be recorded in triplicate between 1-4h after the start of the meal.
	17. Analysis of ex-vivo samples obtained in Parts A and B is optional, and can be limited to only those from select	

Adaptive Study Design Areas	Features	Limits
	dose levels or not done at all. If PK sampling times are changed then the assay sampling times will be changed.	
	18. Exploratory analysis of post-dose serum folate levels may be performed on selected or all dose levels. 19. Exploratory analysis of RBC folate samples may be performed on selected or all dose levels.	
	20. Exploratory analysis of CYP isoforms and transporters may be performed on selected or all dose levels. 21. The CYP isoforms and transporters analysed can be adjusted according to emerging data.	

The study will be performed in two parts: (A) double-blind randomised, placebo-controlled, parallel group, single ascending dose (SAD) in healthy men and WNCBP under fasted conditions; (B) food effect in an open-label, randomised fed/fasted crossover design. The study will be conducted as an adaptive integrated design. Dose escalation to a consecutive higher exposure dosing regimen or to Part B will only occur after satisfactory review of all safety, tolerability and PK data by the Safety Review Committee (SRC) in accordance with the Adaptive Features (

Table 1), rules for dose escalation/progression and toxicity.

Part A (SAD)

In Part A, the safety, tolerability and pharmacokinetics of single ascending doses of P218 will be studied in healthy men and WNCBP. Part A is a double-blind randomised, placebo-controlled, parallel group, ascending dose study and will comprise up to eight fasted cohorts (8 volunteers in each) that will receive a single, ascending dose (SAD) of P218. Within each cohort 6 subjects will receive a single dose of P218 and 2 subjects will receive a single dose of matching placebo (3:1 ratio).

Subjects will be screened within 28 days prior to entering the study on Day -1. Each subject will receive verbal and written information followed by signing of the Informed Consent Form (ICF) prior to any screening procedures taking place. Subjects will be admitted to the study unit on Day-1 and will be discharged by Day 4. All subjects will attend the unit for an outpatient visit on Days 6, 8, 9 and a follow-up visit 11 days post-dose. All the assessments performed during the study are detailed in the study schedule of assessments (Table 2, Table 3, Table 4, Table 5). Study design features may be adapted according to the Adaptive Features (

Table 1).

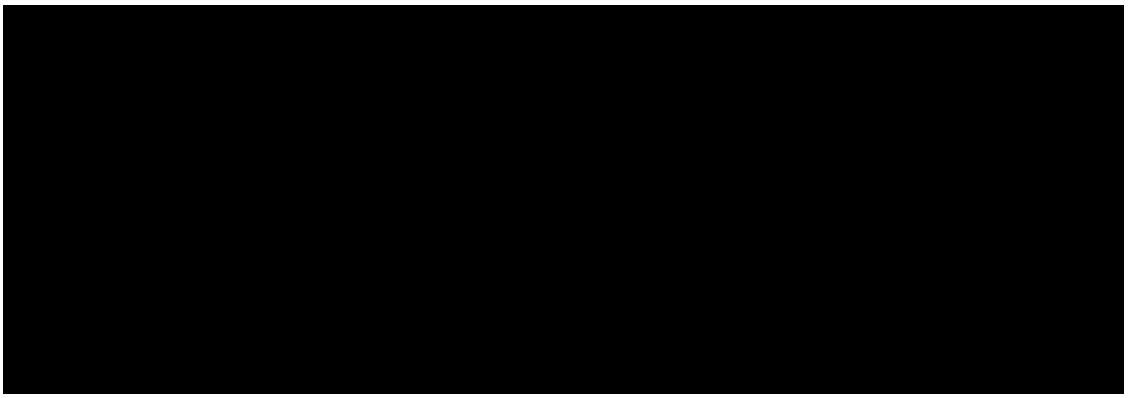

Figure 1. Part A flow chart.

Part B (Food effect)

For the pilot food effect evaluation, a separate cohort of 8 subjects will receive a P218 dose associated with the targeted efficacious exposure (in human subjects in fasted conditions) in an open-label fashion at two occasions separated by a minimal washout of 5 x T1/2 (that will not exceed 3 weeks). The treatment conditions (fed-fasted) will be randomized according to a two-way crossover design. For the fed period, a standard high fat breakfast is given as per FDA guidance; the breakfast is given 30 minutes prior to the scheduled dosing time, and should be completed 10 minutes before dosing (http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126833.pdf).

Figure 1. Part B flow chart

Table 2. Study Plan – Part A: Single Ascending Dose

Study day	**Screening -28 to -2**	**Admission -1**	**Pre-dose**	**1**										**2**	**3**	**4**	**6**	**8**	**9**	**11**
Time point (h)				**0h**	**0.25**	**0.5**	**1**	**2**	**4**	**6**	**7**	**8**	**12**	**24**	**48**	**72**	**120**	**168**	**192**	**240**
Informed consent	X																			
Demographic data	X																			
Medical history	X	X																		
Social history: Smoking, alcohol, drugs status	X																			
Alcohol breath test & urine drug screen	X	X																		
Eligibility criteria assessment	X	X																		
Body Height	X																			
Body Weight	X	X																		X
BMI	X																			
FSH for confirmation of hormonal status	X																			
Serum Pregnancy test	X																			
Serology	X																			
Safety haematology, biochemistry, coagulation	X	X												X [a]		X [a]				X
Serum folate levels + RBC folate levels	X	X												X		X				X
Pharmacogenetic samples			X																	
Physical examination	X	X	X[b]											X[b]						X[b]
Vital Signs[c]	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12 lead ECGs (triplicates)	X	X	X[d]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis	X	X	X											X		X				X
Dosing				X																
PK blood collection			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PK urine collection			X	X →										→	X					

Study day	**Screening -28 to -2**	**Admission -1**	**Pre-dose**	**1**										**2**	**3**	**4**	**6**	**8**	**9**	**11**
Time point (h)				**0h**	**0.25**	**0.5**	**1**	**2**	**4**	**6**	**7**	**8**	**12**	**24**	**48**	**72**	**120**	**168**	**192**	**240**
Telemetry [e]			X	→	→	→	→	→	→	→	→	→	X							
Adverse event assessment				X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	X
Concomitant medication	X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	X
In-house		X	→	→	→	→	→	→	→	→	→	→	→	→	X					
Discharge																X				
Standard Meals[f]		X	→	→	→	→	→	→	→	→	→	→	→	→	→	X				
Malaria bioassay blood collection			X		X		X	X		X			X	X	X		X			X

[a] Haematology and biochemistry only.

[b] Brief physical examination.

[c] Vital signs includes supine systolic and diastolic blood pressure, heart rate and tympanic temperature. Standing blood pressure will be measured at Screening, pre-dose, 2 and 8 hours post-dose.

[d] Three triplicate ECGs to be done at -2, -1 and -0.5 hours pre-dose for the purpose of intensive ECG monitoring.

[e] Telemetry is to be measured from between a minimum of 1 hour pre-dose up to 12 hours post dose.

[f] Lunch will be provided 4 hours after dosing. Dinner will be provided approximately 9 to 10 hours after dosing. An evening snack will be permitted. While confined, the total daily nutritional composition should be approximately 50% carbohydrate, 35% fat and 15% protein. The daily caloric intake per subject should not exceed approximately 3200 kcal.

Table 3. Study Plan – Part A (continuation)

Study Day	Time (h)	PK blood sampling	PK urine sampling[A]	Vital signs[B]	12-lead ECG[C]	Malaria Bioassay
D1	-2				X	
	-1				X	
	-0.5	X		X	X	X
	0		X			
	0.25	X				X
	0.5	X		X	X	
	1	X		X	X	X
	2	X		X	X	X
	4 [D]	X		X	X	
	6	X	X	X	X	X
	7	X		X	X	
	8	X		X	X	
	12	X	X	X	X	X
D2	24	X	X	X	X	X
D3	48	X	X	X	X	X
D4	72	X		X	X	
D6	120	X		X	X	X
D8	168	X		X	X	
D9	192	X		X	X	
D11	240	X		X	X	X

A: Pooled urine collection at 0-6, 6-12, 12-24 and 24-48 hours.
B: Vital signs will be measured after rest of at least 10 min in supine position.
C: 12-lead ECG will be measured in triplicate after a rest period of at least 10 min.
D: Lunch.

Table 4. Study Plan – Part B: Food effect

		Period 1 and Period 2													
Study day	**Screening -28 to -2**	**Admission -1**	**Pre-dose**	**1**								**2**	**3**	**4**	**6**
Time point (h)				**0h**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**120**
Informed consent	X														
Demographic data	X														
Medical history	X	X													
Social history: Smoking, alcohol, drugs status	X														
Alcohol breath test & urine drug screen	X	X													
Eligibility criteria assessment	X	X													
Body Height	X														
Body Weight	X	X													X
BMI	X														
FSH for confirmation of hormonal status	X														
Serum Pregnancy test	X														
Serology	X														
Safety haematology, biochemistry, coagulation	X	X										X [a]		X [a]	X
Serum folate levels + RBC folate levels	X	X										X		X	X
Pharmacogenetic samples			X												
Physical examination	X	X	X [b]									X [b]			X [b]
Vital Signs[c]	X	X	X			X	X	X	X	X	X	X	X	X	X
12 lead ECGs (triplicates)	X	X	X			X	X	X	X	X	X	X	X	X	X
Urinalysis	X	X	X									X		X	X
Dosing				X											
PK blood collection			X		X	X	X	X	X	X	X	X	X	X	X

		Period 1 and Period 2													
Study day	**Screening -28 to -2**	**Admission -1**	**Pre-dose**	**1**								**2**	**3**	**4**	**6**
Time point (h)				**0h**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**120**
Malaria bioassay blood collection			X		X	X	X	X	X	X	X	X	X	X	X
Adverse event assessment				X→	→	→	→	→	→	→	→	→	→	→	X
Concomitant medication	X→	→	→	→	→	→	→	→	→	→	→	→	→	→	X
In-house		X→	→	→	→	→	→	→	→	→	→	→	X		
Discharge														X	
Standard Meals[d]		X→	→	→	→	→	→	→	→	→	→	→	→	X	
Meal record		X→	→	→	→	→	→	→	→	→	→	X			

[a] Haematology and biochemistry only.

[b] Brief physical examination.

[c] Vital signs includes supine systolic and diastolic blood pressure, heart rate and tympanic temperature. Standing blood pressure will be measured at Screening, pre-dose, 2 and 8 hours post-dose.

[d]. For the fed period, a standard high fat breakfast is given as per FDA guidance; the breakfast is given 30 minutes prior to the scheduled dosing time, and should be completed 10 minutes before dosing.

Table 5. Study Plan – Part B (continuation)

Study Day	Time (h)	PK blood sampling	Malaria Bioassay	Vital signs[A]	12-lead ECG[B]	High fat breakfast
D1	-0.5	X	X	X	X	X
	0					
	0.25	X	X			
	0.5	X	X	X	X	
	1	X	X	X	X	
	2	X	X	X	X	
	4	X	X	X	X	
	8	X	X	X	X	
	12	X	X	X	X	
D2	24	X	X	X	X	
D3	48	X	X	X	X	
D4	72	X	X	X	X	
D6	120	X	X	X	X	

A: Vital signs will be measured after rest of at least 10 min in supine position.
B: 12-lead ECG will be measured in triplicate after a rest period of at least 10 min.

8.2 Rules for dose escalation/progression

A safety review committee (SRC) will review and assess at least the minimum required data from the previous cohort(s) to make decisions on the dose(s) for the next dose levels. The SRC will assess if any of the group or individual toxicity criteria are met and if the concerned AEs have a causal relationship with the IMP. If so, the SRC will take action in accordance with the rules stipulated in section 8.2.

There is an option to have ad-hoc SRC meetings to discuss urgent issues should the need arise.

8.2.1 Safety Review Committee

The SRC will consist of, as a minimum:

- MMV Medical Director or delegate (voting member)
- Principal Investigator (RPL) or delegate (voting member)

MMV Project Director or further internal or external experts such as a pharmacokineticist and/or a statistician may be consulted by the SRC as necessary. Any additional information, if required will be included in the SRC charter, located in the study operations manual (SOM).

8.2.2 Data requirements

After each cohort the SRC will assess the minimum safety, tolerability and PK data required prior to dose escalation/progression (refer to section 8.2.2). Prior to each SRC meeting, an interim safety report will be prepared for that cohort presenting the relevant safety and tolerability data. These will be signed by the PI and/or delegate. PK data will be provided separately by the analytical laboratory. As the initial data review will be blinded, there will be no link between the safety data and the PK data.

The following data will be required:

<u>Safety:</u>

- AEs;
- Vital sign parameters (temperature, blood pressure, heart rate);
- 12-lead ECGs and telemetry recordings;
- Clinical laboratory parameters (hematology, coagulation, biochemistry and urinalysis).

<u>Pharmacokinetic data</u>

For availability of PK data, refer to section 8.2.4 below.

<u>Additional data that may be available at the time of report writing:</u>

- Other relevant clinical tests conducted on a case-by-case basis.
- Other exploratory samples.

8.2.3 SRC Meeting

The SRC will determine dose escalations/progression according to the Clinical Study Protocol (CSP), anticipated doses, adaptive features and group toxicity rules. The decision will be signed by one of the Sponsor's representatives and by the PI or delegate. For logistical reasons, the signature of the Sponsor or Investigator may be communicated via email.

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

The decision of the SRC on the next dose will be taken in consensus between the members of the SRC. If consensus cannot be reached then the most cautious approach will proceed. The decisions and decision-making of the SRC on the next dose level will be documented and provided to the PI and Pharmacist prior to the next scheduled dosing day.

8.2.4 Dose escalation/progression

Dose escalation/progression is defined as either of the following:

1. Escalation to a higher exposure dosing regimen within Part A (SAD) cohorts.
2. Progression from Part A (SAD) to Part B (food effect evaluation) cohort.

Minimum data requirements for dose escalation/progression are:

1. Escalation to a higher exposure dosing regimen within Part A (SAD) cohorts.

A minimum of 168 hours post-dose safety and PK data from a minimum of 6 subjects (of which a minimum of 4 will have received the active compound) from the cohort with the next lower exposure level will be required.

2. Progression from Part A (SAD) to Part B (food effect evaluation).

A minimum of 168 hours post-dose safety and PK data from a minimum of 6 subjects (of which a minimum of 4 will have received the active compound) from a previous Part A cohort on a relevant dose level will be required.

"Relevant dose level" is defined as the dose that demonstrated acceptable safety/tolerability at a mean exposure which is not anticipated to be exceeded by the dosing regimen in Part B, taking into account a potential food effect of 3-fold.

8.3 Toxicity rules

For the purpose of this CSP:

1. Toxicity means clinically significant and at least possibly drug related adverse reaction(s).

Table 6. WHO Toxicity Grading

WHO intensity grades are defined as follows:	
1	Mild – No interference with routine activity.
2	Moderate – Interferes with performance of some activities of daily living, but responds to symptomatic therapy or rest.
3	Severe – Significantly limits ability to perform activities of daily living despite symptomatic therapy.
4	Very severe – Incapacitates patient despite symptomatic therapy; requires hospitalization.

WHO Handbook for Reporting Results of Cancer Treatment. WHO Offset Publication No. 48. World Health Organization; Geneva, Switzerland. 1979:16–21.

For more detailed documentation on WHO Toxicity Grading see Appendix A

8.3.1 Group toxicity rules (Part A and Part B)

Table 7. Group Toxicity Rules for progression to Part B and/or escalation to higher exposure dosing regimens in part A.

			Number of subjects		**Toxicity Rules for progression to part B /or escalation to higher exposure dosing regimens within part A**	
WHO 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 part B and/or escalation to higher exposure dosing regimens in part A on hold until results of full (or extended) dosing regimen are available, to which toxicity rules will be applied. Progression/escalation can then proceed, unless the data meet suspension rules.
			≥3	≥4	Yes	Progression to lower exposure dosing regimens is permitted. Progression to part B and/or escalation to higher exposure dosing regimens require substantial amendment.

			Number of subjects		Toxicity Rules for progression to part B /or escalation to higher exposure dosing regimens within part A	
WHO Grade	**Severity**	**Seriousness**	**Number of subjects affected in one SOC**	**Total Number of subjects affected**	**Showing signs of reversibility within 7 days**	**Action**
			N/A	1	No	Progression to part B and/or escalation to higher exposure dosing regimens in part A on hold until results of full (or extended) dosing regimen are available, to which toxicity rules will be applied. Progression/escalation can then proceed, unless the data meet suspension rules.
			N/A	≥2	No	Progression to lower exposure dosing regimens is permitted. Progression to part B and/or escalation to higher exposure dosing regimens require substantial amendment.
3	Severe	Not serious	N/A	1	Yes	Progression to part B and/or escalation to higher exposure dosing regimens in part A on hold until results of full (or extended) dosing regimen are available, to which toxicity rules will be applied. Progression/escalation can then proceed, unless the data meet suspension rules.
			N/A	≥2	Yes	Progression to lower exposure dosing regimens is permitted. Progression to part B and/or escalation to higher exposure dosing regimens require substantial amendment.
			N/A	≥1	No	
3	Severe	Serious	N/A	≥1	N/A	
4	Very Severe	Serious	N/A	≥1	N/A	Study suspended

Table 8. Group Toxicity Rules for continuation within a dosing regimen.

			Number of subjects		Toxicity Rules for continuation within a dosing regimen	
WHO Grade	**Severity**	**Seriousness**	**Number of subjects affected in one SOC**	**Total Number of subjects affected**	**Showing signs of reversibility within 7days**	**Action**
1	Mild	N/A	Any	Any	N/A	No action required
2	Moderate	N/A	2	≤4	Yes	Dosing of the remainder of the dosing regimen can continue as per CSP.
			≥3	≥5	Yes	Dosing of the remainder of the dosing regimen suspended; Continuation requires substantial amendment
			N/A	1	No	Dosing of the remainder of the dosing regimen can continue as per CSP.
				≥2	No	Dosing of the remainder of the dosing regimen suspended; Continuation requires substantial amendment
3	Severe	Not serious	N/A	1	Yes	Dosing of the remainder of the dosing regimen can continue as per CSP.
				≥2	Yes	Dosing of the remainder of the dosing regimen suspended; Continuation requires substantial amendment
				≥1	No	
3	Severe	Serious	N/A	≥1	N/A	
4	Very Severe	Serious	N/A	≥1	N/A	Study suspended

8.3.2 Individual toxicity rules (Part B only)

Table 9. Individual toxicity rules.

			Individual toxicity rules	
WHO Grade	**Severity**	**Seriousness**	**Showing signs of reversibility within 7 days**	**Action**
1	Mild	N/A	N/A	No action required
2	Moderate	N/A	Yes	IMP administration may be continued, amended, temporarily suspended or discontinued in accordance with Investigator's clinical judgement and relevant algorithms for the treatment of toxicities
			No	IMP administration will be discontinued
3	Severe	Not serious	N/A	IMP administration will be discontinued
3	Severe	Serious	N/A	IMP administration will be discontinued
4	Very Severe	Serious	N/A	IMP administration will be discontinued

Standard toxicity grading (according to the WHO Toxicity Grading) will be used to grade the AEs for the purpose of applying the toxicity rules. This owes to the fact that this toxicity grading is more relevant to the population which P218 is aiming to treat.

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.

Non-clinical data as well as potential class effects have identified the risks outlined in section 6.3.2.1 (GI toxicity).

The grading of the WHO criteria related to toxicities and potential AEs (including study related sections on: 'stomatitis', 'nausea' and 'vomiting', 'diarrhoea' and 'constipation') is considered suitable for this particular IMP, study design and the study populations in conjunction with the CSP specific toxicity and dose progression/escalation rules. No further qualifications are required.

8.3.3 PK exposure limit

In addition to the above rules, progression and escalation will also be limited by PK exposure limits.

14-day GLP toxicology studies conducted in rat and dogs have shown that dog is the most sensitive species with the lowest dose tested (10mg/kg/day) associated with reversible microscopic findings in the gallbladder. Studies in both rats and dogs showed GI tract toxicity that was consistent between species and reflected consequence of DHFR inhibition and depletion of folate. Supplementation of P218 with folinic acid in the rat abolished all GI pathology confirming this to be related to chronic DHFR inhibition. Single dose administration of P218 did not induce any GI tract toxicity in animals.

Based on the high exposures achieved at the rat NOAEL (40mg/kg/day) and the ability to monitor the observed toxicity (primarily GI) in the dog at doses > 10mg/kg/day, the dog exposure achieved at the next dose level in dogs (20mg/kg/day – 71.9µg.hr/mL) is identified as the highest acceptable human exposure for this FIM study with single dose administration of P218 to healthy subjects. The corresponding unbound exposure is 15.5µg.hr/mL)

71. 9µg.hr/mL is the cumulative AUC over the 14 days of the dog study. As subjects will receive only a single dose of P218, it is considered that mean exposure in human, AUC_{last} is equivalent to $AUC_{0\text{-}336}$ PK derived exposure limit in the 14 day toxicology study with dogs.

In this FIM study, preliminary PK of each dose level in Part A will be analyzed after completion of each cohort to ensure that the mean group exposure of the subsequent cohort will not exceed the highest achievable human exposure (71.9µg.hr/mL).

8.4 Rationale for study design, doses and control groups

This study is the first time that P218 will be given to humans.

Part A

A single dose escalation ascending dose design is used for this study to evaluate the safety, tolerability and PK of the P218 in healthy male and female subjects of non-child bearing potential. The study is randomised and double blinded to minimise bias and includes placebo to facilitate identification of effects related to administration of drug rather than the study procedures or situation (Part A).

Part B

There will be no placebo as this is a cross-over design to investigate the food effect on the safety, tolerability and PK of P218. Each subject will be their own control, and therefore minimise inter-subject bias and allow identification of effects related to the adminstration of drug with food rather than the study procedures or situation.

8.4.1 Estimation of first dose

The maximum recommended safe starting dose (MRSD) of 10mg was calculated based on results from pre-clinical studies and in accordance with the 2005 FDA Guidance (Guidance for Industry - Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Subjects, FDA 2005) (1) and the 2007 EMEA Guidance EMEA/CHMP/SWP/28367/07 (2).

The NOAEL in the 14-day rat toxicology study was 40mg/kg/day, while the corresponding value in the 14-day dog toxicology study was <10mg/kg/day (due to minimal reversible microscopic gallbladder lesions in male animals at the lowest dose tested). On the basis of body surface area, the corresponding extrapolated human equivalent doses for a human body weight range of 50-70kg were 322-451mg (rat NOAEL) and 277-388mg (dog exposure at 10mg/kg/day), respectively. Utilizing the lower of the two calculated doses (human equivalent dose of 277-388 mg on the basis of the dog data) and applying a safety factor of 10, a starting dose of 28 mg could be supported from a toxicology perspective. As the lowest dose tested in dogs was

associated with reversible microscopic findings in the gallbladder (in male dogs only), a conservative approach is used and a human starting dose of 10mg is identified for this protocol.

This proposed starting dose of 10mg was predicted to generate mean C_{max} and AUC values of 0.220µg/ml and 1.27µg·h/ml, respectively in the most conservative scenario from a safety point of view (lowest human predicted clearance, highest exposure). These estimates provide a predicted safety margin of 4.2- and 34.7-fold when compared to the C_{max} (0.921µg/ml) and cumulative AUC_{0-336} (44.1 µg·h/ml) in the most sensitive species (dog) at 10mg/kg/day. When compared to unbound C_{max} (0.198µg/ml) and unbound cumulative AUC_{0-336} (9.48µg·h/ml), the margins increase to 15.2 and 124.7 respectively.

The Predicted unbound Cmax at starting dose (10 mg) is 13 ng/mL. Minimal inhibition on the human DHFR should occur when the free concentration of P218 reaches IC10 (51.1 ng/ml). This represents a 3.9-fold safety margin.

The table below indicates the margins between the starting dose (and the subsequent clinical doses) and the following value: 44.1 µg.h/mL, which represents the AUC0-336 reached at 10 mg/kg/day (dose with minimal toxicology effect – close to NOAEL).

Table 7 Margins between the clinical doses predicted AUC_{inf} and the 10 mg/kg/day AUC0-336 in the dog

If low Cl = 0.7 ml/min/kg				
Dose (for 70 kg)	AUC_{inf} (µg.h/ml)	Cum $_{AUC0-336}$ margin	Unbound AUC_{inf} (µg.h/ml)	Unbound cum AUC_{0-336} margin
10	1.27	34.72	0.08	118.5
30	3.82	11.54	0.23	41.22
100	12.71	3.47	0.76	12.47
250	31.82	1.39	1.91	4.96
500	63.60	0.69	3.82	2.48
1000	127.20	0.35	7.63	1.24
1500*	190.80	0.23	11.45	0.83
2000*	254.43	0.17	15.27	0.62

*- Dose not administered.

The table below indicates the margins between the starting dose (and the subsequent clinical doses) and the following value: 35.7 ug/mL, which represents the highest C_{max} observed in animal, i.e. single dose of 2000mg/kg in rat (35.7 ug/mL).

Table 8 Margins between the clinical doses predicted C_{max} and the highest C_{max} observed in animals

If low Cl = 0.7 ml/min/kg				
Dose (for 70 kg)	C_{max} (µg/mL)	C_{max} margin	Unbound C_{max} (µg/mL)	Unbound C_{max} margin
10	0.22	162.3	0.013	302.1
30	0.66	54.1	0.040	98.2
100	2.20	16.2	0.132	29.8
250	5.52	6.5	0.331	11.9
500	11.04	3.2	0.662	5.9
1000	22.07	1.6	1.324	3.0
1500*	33.11	1.1	1.987	2.0
2000*	44.16	0.8	2.649	1.5

*- Dose not administered.

8.4.2 Justification of the planned highest dose level

Efficacy

Preclinical studies using SCID mice inoculated with *P. falciparum*-infected red blood cells link doses and exposures to the efficacy of P218. The active dose in the SCID model causing maximum effect (ED_{90}) against the parasites is 1.6 mg/kg/day. The minimal parasiticidal concentration (MPC) and the minimal inhibitory concentration (MIC) derived from the SCID data are 4.4 ng/ml and 1.3 ng/ml, respectively.

A human dose that could maintain blood concentrations above MIC for seven days was identified as a target minimal efficacious dose for the chemoprevention of Pf malaria. With this approach and the preliminary PK data collected during the FIH cohorts 1 to 6, it is likely that a human dose ≥ 750 mg may be needed in malaria patients to maintain P218 plasma concentration above mouse blood MIC.

Due to cost limitations associated with Malaria prevention in endemic countries, 1 g is considered the highest feasible dose which would be economically and technically sustainable, and therefore 1g is the highest dose that we intend to investigate. The predicted P218 exposure at this dose is 16.5 µg•h/mL and the resulting margin to P218 AUC cap is 4.4 folds.

Toxicology

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 (preliminary QCed results). Given that the structure, and in particular the site of conjugation, is identical between both acyl glucuronides of P218; it is considered appropriate to assess safety in terms of total acyl glucuronide burden rather than consider each one independently.

The toxicology coverage of human P218 β acyl glucuronide and P218-OH β acyl glucuronide will therefore be assessed by considering both molecules as a whole, with the global human

AUC being the sum of the human AUC_{last} of each molecule. 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. The predicted P218 β acyl glucuronides global human AUC at a 1000 mg dose (based on linear extrapolation to be 48.2 μg•h/mL) is projected to remain below the highest tolerated, estimated cumulative glucuronides AUCs in the dog 14 day toxicology study (74.6 μg•h/mL) and the rat folate supplementation study (62.5 μg•h/mL). In addition, as indicated above, at this dose, P218 should also remain below the AUC cap of 71.9μg•h/mL. Thus, it is considered safe to perform a 1000 mg dose cohort in the FIH study.

8.4.3 Choice of subjects for study

The target population of patients with malaria will not be included. Since reproductive toxicology data are not available before study initiation, the study will be performed in males and WNCBP.

8.4.4 Route and rate of administration

P218 will be administered via the oral route, as this is the most appropriate for the target population. In this study, planned dose levels were calculated from pre-clinical data. Dose escalation will proceed with accordance with section 8.28.2 and

Table 1 and will not exceed the mean maximum exposure limits.

8.4.5 Precautions to be applied for dosing between subjects within a cohort

For each cohort, treatment of subjects will begin on Day 1. Subjects will be randomised on Day 1 at each dose level. A mandatory sentinel dosing strategy of dosing 2 subjects (1 subject on IMP, 1 subject on placebo) will be used for the first three cohorts in Part A. If safety and tolerability is acceptable, the remaining subjects of this cohort may be dosed, with a minimum interval of 24 hours between the first subject of the sentinel group and the remaining subjects of that dose level being dosed.

The Investigator will make a judgement (based on the clinical data available at the time) whether the drug administration to the remaining subjects in the cohort can continue. The assessment will be based on the clinical safety data available at the time and the protocol toxicity rules (Section 8.3). The Investigator will document the decision in an e-mail to the Sponsor. The e-mail does not require Sponsor's response, unless there is disagreement with the Investigator's decision.

Sentinel dosing for all other study parts is not considered necessary as the dose escalation and progression rules, PK exposure limits and toxicity rules provide appropriate rules and boundaries to ensure safety.

8.4.6 Precautions to be applied for dosing between different cohorts

Progression between cohorts will be determined by the SRC according to the anticipated doses, Adaptive Features (

Table 1) and group toxicity rules (Section 8.3.1). Safety and PK data will be continuously evaluated in line with the dose progression rules and minimum data requirements. Please refer to section 8.2.

8.4.7 Monitoring and communication of adverse events / reactions

Adverse events (AEs) will be continuously monitored throughout the study from first dose until the last follow up assessment. Each AE reported will be assessed by a trained Research Physician who will ensure that the event is dealt with as appropriate based on clinical need, study protocol, study operations manual and Richmond Pharmacology standard operating procedures (SOPs). AEs will be documented in the subjects' Case Report Forms (CRFs) and reviewed regularly by the Research Physicians and the Investigator.

If any information relating to the study drug in this study becomes available after the submission of a final protocol to the Competent Authority which may impact on the conduct of the study, including but not limited to the risk and benefit evaluations underpinning approvals and volunteer's consent, MMV shall notify RPL in writing as soon as practically possible and the parties will agree, in writing, what steps need to be taken if any.

8.4.8 Investigator Site Facilities and Personnel

This study will be conducted in a specialised early phase CPU within an acute hospital setting with Critical Care facilities, thus ensuring direct access to equipment and staff for resuscitating and stabilising subjects in acute medical conditions and emergencies. The study is conducted by an experienced PI and well trained medical, nursing and technical staff with ample experience in the conduct of early phase clinical trials.

The study is designed to closely monitor, treat and communicate potential expected adverse reactions (based on the known mode of action of the IMP and the previous studies with similar compounds) as well as potential unexpected adverse events.

A specialist medical advisor will be available to provide input to the PI and Sponsor on any gynaecological queries.

9. SELECTION AND WITHDRAWAL OF SUBJECTS

9.1 Number and Source of Subjects

9.2 Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for enrolment in this study:

1. Subject is a healthy male or female (of non-childbearing potential), aged 18 to 45 years, inclusive.

2. Satisfactory medical assessment with no clinically significant or relevant abnormalities as determined by medical history, physical examination, vital signs, 12-lead ECG, and clinical laboratory evaluation (haematology, biochemistry, coagulation, and urinalysis) that is reasonably likely to interfere with the subject's participation in or ability to complete the study as assessed by the Investigator.

3. Subject has a body weight of at least 50 kg and a body mass index (BMI) of 18-25 Kg/m2, inclusive.

4. Female subjects must be of non-childbearing potential:

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

5. Heterosexually active male subjects with a female spouse/partner of childbearing potential must agree to use barrier contraception (male condom), even with documented medical assessment of surgical success of a vasectomy, if your partner could become pregnant from the time of the first administration of P218 and for 100 days following this. Your partner must also use a method of highly effective contraception including:

    - Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Intravaginal
        - Transdermal
    - Progestogen-only hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Injectable
        - Implantable
    - Intrauterine device
    - Intrauterine hormone-releasing system
    - Bilateral tubal occlusion

6. 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.

7. Ability to swallow multiple capsules at a time or (consecutively) 1 capsule at a time.

8. Subjects must be capable of fully understanding and complying with the requirements of the study and must have signed the informed consent form prior to undergoing any study-related procedures.

9. Subjects who are willing and able to comply with all scheduled visits, treatment plan, laboratory tests, and other study procedures.

9.3 Exclusion criteria

Subjects will be prohibited from participating in this study if they meet any of these criteria:

1. Male subjects with a female partner(s) who is (are) pregnant or lactating from the time of the administration of study medication.

2. Women of childbearing potential, defined as all women physiologically capable of becoming pregnant, including women whose career, lifestyle, or sexual orientation precludes intercourse with a male partner and women whose partners have been sterilized by vasectomy or other means.

3. Evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal (including gallbladder), cardiovascular, hepatic, psychiatric, neurologic, or allergic disease (including drug or food allergies, anaphylaxis or other severe allergic reactions but excluding untreated, asymptomatic, seasonal allergies at the time of dosing).

4. Current or relevant history of physical or psychiatric illness that may require treatment or make the subject unlikely to fully comply with the requirements or complete the study, or any condition that presents undue risk from the investigational product or study procedures.

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

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

7. Any other significant disease or disorder which, in the opinion of the investigator, may either put the subject at risk because of the participation in the study may influence the result of the study, or the subject's ability to participate in the study.

8. History of photosensitivity.

9. History of megaloblastic anaemia or folate deficiency.

10. History or clinical evidence of substance and/or alcohol abuse within the 12 months before screening. Alcohol abuse is defined as regular weekly intake of more than 21 units for males and 14 units for females (using alcohol tracker http://www.nhs.uk/Tools/Pages/NHSAlcoholtracker.aspx).

11. Treatment with an investigational drug within 90 days or 5 half-lives preceding the first dose of study medication (or as determined by the local requirement, whichever is the longer).

12. Donation of blood or blood products (excluding plasma) within 90 days prior to study medication administration.

13. Use of moderate/strong inhibitors or inducers of CYP450 cytochromes or transporters within 30 days or 5 half-lives (whichever is the longer) prior to the first dose of study medication.

14. Consumption of grapefruit, grapefruit juice or grapefruit-related citrus fruits (e.g. Seville oranges, pomelos) within 30 days prior to the first dose of study medication.

15. Ingestion of any poppy seeds within the 24 hours prior to screening.

16. Use of prescription or non-prescription drugs and dietary supplements within 7 days or 5 half-lives (whichever is the longer) prior to the first dose of study medication. With the exception of paracetamol, which may be used incidentally or for a short-term treatment at a maximum dose of 1 gr. per day.

17. Use of herbal supplements at least 30 days prior to the first dose of study medication.

18. Any clinically significant abnormal laboratory, vital signs or other safety findings as determined by medical history, physical examination or other evaluations conducted at screening or on admission.

19. The history or presence of any of the following cardiac conditions: known structural cardiac abnormalities; family history of long QT syndrome; cardiac syncope or recurrent, idiopathic syncope; exercise related clinically significant cardiac events.

    - Any clinically important abnormalities in rhythm, conduction or morphology of resting ECG that may interfere with the interpretation of QTc interval changes. This includes subjects with any of the following (at screening):
    - Sinus node dysfunction.
    - Clinically significant PR (PQ) interval prolongation.
    - Intermittent second or third degree AV block.
    - Incomplete or complete bundle branch block.
    - Abnormal T wave morphology.
    - Prolonged QTcB >450 ms or shortened QTcB < 350 ms. Any other ECG abnormalities in the standard 12-lead ECG and 24-hour 12 lead Holter ECG or an equivalent assessment which in the opinion of the Investigator will interfere with the ECG analysis.

    Subjects with borderline abnormalities may be included if the deviations do not pose a safety risk, and if agreed between the appointed Cardiologist and the PI.

20. Confirmed positive results from urine drug screen (amphetamines, benzodiazepines, cocaine, cannabinoids, opiates, barbiturates, and methadone) or from the alcohol breath test at screening or on admission.

21. A positive human immunodeficiency virus (HIV) I and II antibodies, hepatitis B surface antigen (HBsAg), anti Hepatitis core antibody (anti HBc Ig G [and anti HBc IgM if IgG is positive], or hepatitis C virus (HCV) antibody at screening.

22. Subjects have veins unsuitable for intravenous puncture or cannulation on either arm (e.g. veins that are difficult to locate access or puncture veins with a tendency to rupture during or after puncture).

23. Any conditions which in the opinion of the investigator would make the subject unsuitable for enrolment or could interfere with the subjects' participation in or completion of the study.

9.4 Subject Restrictions

Subjects will have to comply with the following restrictions during the study:

1. Whenever subjects are confined in the ward, only the drinks and meals provided by the trial personnel will be allowed.
2. Standard meals will be provided at the standard unit times as stated in the study plan, and meals should be completed each time.
3. Subjects will be required to fast for a period of at least 4 hours prior to laboratory safety tests on screening and the post/study visit days. On Day 1 of study Part B will receive breakfast before study drug administration. On all other dosing days, subjects will fast for at least 8 hours pre-dose.
4. Subjects will be provided water ad libitum up to 1 hour pre-dose and from 1 hour post-dose.
5. Abstain from consumption of caffeine from 24 hours prior to screening and study drug administration until collection of the final PK sample.
6. Abstain from consumption of energy drinks containing taurine or glucuronolactone from 24 hours prior to screening and study drug administration until collection of the final PK sample.
7. Abstain from eating or drinking grapefruit or grapefruit-related citrus fruits (e.g. Seville oranges, pomelos) within 30 days prior to the first dose of study medication until collection of the final pharmacokinetic blood sample.
8. Abstain from drinking alcohol from 24 hours prior to screening and study drug administration until collection of the final PK sample. Subjects may undergo an alcohol breath test at the discretion of the investigator.
9. Abstain from smoking and/or using nicotine containing products for 24 hours prior to study drug administration and throughout confinement in research unit.
10. Abstain from any new or unaccustomed strenuous physical activity or any excessive strenuous activity (e.g. heavy weight lifting, long distance running) for at least 48 hours prior to screening and study drug administration until collection of final PK sample.
11. Abstain from blood and plasma donation during the study and up to three months after completion of the study.

9.5 Subject Inclusion and Randomisation

Subjects in this study will be assigned to a treatment regimen according to a randomization schedule generated by a statistician using PROC Plan. Details regarding the unique screening and subject number will be included in the SOM.

Subjects who meet the eligibility criteria will be randomly assigned on Day 1. In Part A subjects will be randomly assigned in a 3:1 ratio to either P218 or placebo. In Part B, all subjects will receive P218.

9.6 Criteria for withdrawal

The Investigator or designee may withdraw a subject from the study if the subject:

- Is in violation of the protocol;
- Has an AE;
- Meets individual stopping criteria;
- Use of/need for a prohibited medication which in the opinion of the Sponsor or Investigator may jeopardize the study results or represent a risk to the participant;
- Requests to be withdrawn from the study (subject withdrawal of consent);
- Is found to be considerably non-compliant with the protocol-required visits;
- In the Investigator's opinion, is unable to continue study participation;
- Is withdrawn from the study upon the request of Sponsor or the SRC, including if Sponsor terminates the study.

9.6.1 Handling of Withdrawals

In the event a subject withdraws or is withdrawn from the study, the Investigator will inform the Medical Monitor and the Sponsor immediately. The SRC will be notified. If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator for protocol-specified safety follow up procedures.

Should any of the subjects be withdrawn from the study after being dosed, all the relevant assessments in relation to last dose should be completed as per protocol.

Investigator and/or Sponsor may decide to perform additional assessment in accordance with

Table 1 – Adaptive Features.

When a subject withdraws or is withdrawn from the study, every effort should be made to conduct a complete Early Termination (ET) at an appropriate time-point.

A subject who fails to return for final evaluations will be contacted by the site in an attempt to have the subject comply with the protocol in accordance with the site SOPs.

When a subject withdraws from the study, the primary reason for discontinuation must be recorded in the appropriate section of the case report form (CRF).

10. STUDY AND CONCOMITANT TREATMENTS

10.1 Investigational Medicinal Products (IMPs)

The following IMPs will be used in this study:

- P218 as capsule
- Placebo to match P218, as capsule.

P218 will be supplied by Penn Pharma-UK, as capsules.

Due to the favourable physicochemical properties of P218, the 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 10, 50 and 250 mg of P218.

P218 capsules (10, 50 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 Investigator's Brochure (IB) and in the Investigational medicinal product dossier (IMPD).

Placebo capsules will be used for the purpose of the clinical trial. The placebo capsules have been developed to match the drug product. They are contained in the same packaging as the drug product.

Table 9. IMP or matching placebo for each anticipated dose level (Part A)

Treatment:	**IMP Dispensed:**	**Administration**
10 mg P218	1 x P218 10 mg capsule	Oral doses will be administered with 240 mL of water. Subjects will be in the sitting position. For doses of 6 capsules or more subjects will be offered additional water 50 mL x2
30 mg P218	3 x P218 10 mg capsules	
100 mg P218	2 x P218 50 mg capsules	
250 mg P218	1 x P218 250 mg capsule	
500 mg P218	2 x P218 250 mg capsules	
750 mg P218	3 x P218 250 mg capsules	
1000 mg P218	4 x P218 250 mg capsules	
Placebo	Oral dose of placebo to match P218	

Table 10. IMP or matching placebo for the anticipated dose level (Part B)

Treatment:	**IMP Dispensed:**	**Administration**
250 mg P218	1 x P218 250 mg capsule	Oral doses will be administered with 240 mL of water. Subjects will be in the sitting position. If the dose consists of 6 capsules or more subjects will be offered additional water 50 mL x2

The dose will be selected, based on the safety, tolerability, and PK profile of the fasted dose cohorts as the dose corresponding to the predicted human efficacious dose.

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 study site.

10.2 Labelling of IMPs

The labelling of the study drugs will be in compliance with Good Manufacturing Practice (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 UK health authorities according to the submission requirements.

10.3 Drug Accountability

The designated pharmacy staff at the clinical study site will maintain accurate records of receipt and the condition of all study drugs, including dates of receipt. In addition, accurate records will be kept by the pharmacy staff of when and how much study drug is dispensed and used by each subject/patient in the study. Any reason for departure from the protocol dispensing regimen must also be recorded.

Drug accountability records and inventory will be available for verification by the Sponsor or designee. Upon completion of the study, there will be a final reconciliation of all study drugs and copies of study drug accountability records will be provided to MMV. Original records will be maintained at the clinical site with the rest of the study records.

Study drug must not be used for any purpose other than the present study. Remaining study drug will be returned to the Sponsor or its agent or its destruction arranged by the clinical study site according to applicable regulations and only after receipt of written authorization from the Sponsor.

10.4 Blinding and Procedures for Unblinding the Study

10.4.1 Methods for ensuring blinding

The study will be conducted in a double-blind fashion whereby subjects and clinical study site staff are blinded to study drug/dose assignment.

The pharmacy staff preparing the investigational products will not be blinded to study drug assignment. During the study, the randomisation codes will be kept in the site's clinical trials pharmacy, accessible to the pharmacy personnel only. Upon completion of the study, after the database lock and after the blind is revealed, the randomisation list will be filed in the Study Master File.

P218 and Placebo capsules are identical in appearance.

10.4.2 Methods for unblinding the study

In the event of an emergency, an envelope for each subject containing their study drug assignment will be available in the pharmacy at the clinical study site. Unblinding should only be considered for the safety of the subject. If unblinding is deemed necessary by the investigator, the investigator or designee can unblind the subject’s treatment allocation using the envelope available from the pharmacy. The investigator or designee must note the date, time and reason for unblinding and inform the sponsor of unblinding as soon as practicably possible.

10.5 Concomitant Medications/Permitted medications

Intake of any medication is prohibited from 7 days before IMP administration until the end of the hospitalisation in order to avoid interference with study assessments. As an exception, a maximum dose of 1g/day of paracetamol may be used.

During the study, medications other than IMP must only be taken exceptionally and with the consent of the investigator. The need for other medication may lead to subject's withdrawal from the study. In any case, the investigator will inform the sponsor about the concurrent medication given.

Details of prior and concomitant medications should be recorded by the investigator on the CRF and source record.

Emergency equipment and drugs will be available within the clinical unit as per current standard procedures. In the unlikely event that they are required, their use will be documented

11. STUDY PROCEDURES

11.1 Schedule of Study Procedures

The study assessments are described in the sections below and the timing of these assessments are detailed in the Study Plans (Table 2, Table 3, Table 4, and Table 5). It is important that PK sampling occurs as close as possible to scheduled time. The sequence of assessments at particular time-points will be described in the SOM.

12. STUDY METHODOLOGY

12.1 Meals

Standardised meals will be provided during the study period according to the timings described in the Study Plan (Table 2, Table 3, Table 4, and Table 5).

On Day 1 of either, Period 1 or Period 2, of Part B, subjects will have the IMP administered in the fed state. Thirty minutes prior to dosing, the subjects will receive a high-fat breakfast based on that recommended by the FDA, details regarding this will be described in the SOM.

12.2 Haematology, biochemistry and coagulation

Blood samples for determination of biochemistry and haematology parameters will be taken at the times given in the Study Plans (Table 2, Table 3, Table 4, and Table 5). The date and time of collection will be recorded on the appropriate CRF pages. The analyses will be done using routine methods. Further details will be described in the SOM.

Laboratory values outside the reference limits, which are suspected to be of any clinical significance, will be repeated. Subjects in whom the suspected clinical significance is confirmed on repeated sampling will either not be included or, if already included, may be withdrawn from further participation in the study in accordance with the toxicity rules in section 8.3 and/or followed until normalisation or for as long as the Investigator considers necessary.

Laboratory parameters to be measured are presented in Table 11.

Table 11. Laboratory parameters.

Biochemistry	Haematology	Coagulation	Serology	Urinalysis	Urine Screen for Drugs of Abuse
Aspartate aminotransferase	Red blood cell count	Prothrombin time (PT)	Hepatitis B surface antigen (HBsAG)	Leukocytes	Benzodiazepines
	Haemoglobin	International Normalised Ratio (INR)	Hepatitis B core antibody (anti-HBC IgG/IgM + isolated IgM, if IgG positive)	Nitrite	Opiates
	Mean corpuscular volume (MCV)				
	Mean corpuscular haemoglobin concentration (MCHC)				
Alanine aminotransferase	Mean cell haemoglobin (MCH)	Activated partial thromboplastin time (aPTT)	Hepatitis C antibody (anti-HCV)	Urobilinogen	Amphetamines
Alkaline phosphatase	Reticulocytes		HIV I and II antibodies	Protein	Methadone
Blood urea nitrogen	Haematocrit			pH	Cocaine
Gamma glutamyl transferase	Platelets			Blood	Cannabinoids
Total bilirubin Direct bilirubin	White blood cells			Specific gravity	Barbiturates
Creatinine	Neutrophils			Ketones	Benzodiazepines
	Eosinophils			Bilirubin	Opiates
Total serum proteins	Basophils			Glucose	Phencyclidine
Albumin	Lymphocytes			Nitrites	Tricyclic antidepressants
Glucose – fasting	Monocytes			Urine microscopy	Methamphetamine
Sodium					
Potassium					
Calcium					
Magnesium[a]					

Biochemistry	Haematology	Coagulation	Serology	Urinalysis	Urine Screen for Drugs of Abuse
Follicle stimulating hormone[a] Serum pregnancy test Chloride Bicarbonate/CO2 Urate C-reactive protein Globulin Lactate Dehydrogenase Creatine phosphokinase Inorganic phosphate Serum folate					

[a] measured at screening only

12.3 Serology

Serology will be performed at Screening as detailed in the Study Plans (Table 2 and Table 4). At the screening visit all subjects will be tested for the parameters listed in Table 11. This is done for the safety of the study personnel and the result from the tests will not be entered into the study database. If a volunteer is found to be confirmed positive in any of these tests, they will be referred for further examination/treatment and will not be included in the study, with the exception of volunteers with a confirmed positive anti-HBc IgG and negative anti-HBc IgM and HBsAg, indicative of natural immunity due to a past infection without active chronic or acute infection

The serology tests will be analysed in the same blood sample used for biochemistry.

Laboratory results at screening and/or baseline that fall outside of the specified eligibility criteria may be repeated once prior to enrolment and/or randomisation in order to exclude a possible laboratory error. Should the repeat value remain outside of the required range for eligibility, the subject will be excluded from participation.

12.4 Urinalysis

Urine samples for determination of urinalysis parameters will be taken at the times given in the Study plans (Table 2 and Table 4). If deemed necessary, based on a clinically significant positive test, microscopic examination of urine will be performed.

12.5 Pregnancy test

To exclude pregnancy, a serum β-HCG blood sample will be performed as described in Study Plans (Table 2 and Table 4) and whenever pregnancy is suspected. Any subject with a positive pregnancy test will be excluded or withdrawn.

12.6 Drugs of Abuse

Urine will be tested for the drugs of abuse as described in the Study Plans (Table 2 and Table 4). If a subject fails the drug abuse screen, they will be excluded from the study. A repeat drug screen can be done where methodological reasons are believed to have led to a false positive. If subjects are suspected to be positive due to medication e.g. flu/cold remedies, they may undergo a repeat drug screen. The results from the tests will not be entered into the database.

12.7 Alcohol Breath Test

An alcohol breath test will be done using an alcometer as described in the Study Plans ((Table 2 and Table 4)). The results from this test will not be entered into the clinical study database. If a subject tests positive to the test they will be excluded from the study.

12.8 Physical Examination, Height and Weight

The physical examination performed at screening will include an assessment of the following: general appearance, skin, eyes, ears, nose, mouth (including dentition), head, neck (including thyroid), lymph nodes, throat, heart/circulation, chest, lungs, abdomen, musculo-skeletal system, neurological examination and extremities. The timings of the physical examinations are described in the Study Plans (Table 2 and Table 4).

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

Height will be measured in centimetres and weight in kilograms. Measurements should be taken with subjects wearing light clothing and without shoes using calibrated scales for all measurements. BMI will be calculated from the height and weight.

12.9 Vital Signs

12.9.1 Blood pressure, heart rate and tympanic temperature

Blood pressure and heart rate will be measured in supine position after the subject has rested comfortably for at least 5 minutes, followed by the standing position after the subject has stood for 1 minute, using automated Criticon Dynamap® monitors. Vital signs (blood pressure and heart rate) will be measured at the time points as detailed in the Study Plans (Table 2, Table 3, Table 4, and Table 5). Temperature will be measured using tympanic thermometers.

12.10 Electrocardiographic (ECG) Measurements

12.10.1 Recording of 12-lead ECGs

12-lead ECGs will be recorded at the time-points described in Study Plans (Table 2, Table 3, Table 4, and Table 5) using a GE Marquette MAC1200® /MAC1200ST® recorder connected via a fixed network connection to the MUSE® Cardiology Information System (MUSE). ECGs recorded during screening will be stored electronically on the MUSE information system. Only ECG recorded electronically will be valid ECG for any purpose other than safety assessment. ECG printouts may be filed in the subject's CRF for medical safety reviews.

Each ECG recorder will be set up to the required technical specifications and containing the information required to identify the records. Each ECG recording will be clearly identified (Subject ID, visit date, and the actual times of ECG recordings).

12-lead ECG recordings will be made after the subjects have been resting in a supine position for at least 10 minutes. The subjects will avoid postural changes during the ECG recordings and clinical staff will ensure that subjects are awake during the ECG recording. At each time point, triplicate 12-lead ECGs will be recorded.

At each time point, the ECG will be recorded in triplicate, to reduce variance and improve the precision of measurement. The triplicates will be performed at approximately 1-minute intervals. Each ECG recording (trace) will last 10 seconds. Repeat ECG will be performed until at least three 10-second ECG records per scheduled time-point meet the quality criteria set out in the SOM and the applicable SOP so to enable reading and analysing at least 5 complexes per derivation.

12.10.2 Safety review of 12-lead ECGs

All recorded ECGs will be reviewed by a Research Physician and the review be documented in the CRF. With regards to the clinical assessment of QTc interval, QTcB will be assessed as this is automatically calculated by the equipment. If there is any indication of potential significant QTcB prolongation, QTcF will also be calculated and assessed by a Research Physician. If a subject shows an abnormal ECG, additional safety recordings (including the use of 5 or 12 lead Holter equipment) may be made and the abnormality be followed to resolution if required.

Further details will be included in the SOM.

12.10.3 Real time display (ECG telemetry)

A 12-lead real time ECG will be recorded as described in the Study Plan (Table 2, Table 3). ECG telemetry will be monitored by the Investigator or qualified member of clinical staff. This will allow the extraction of ten-second 12-lead ECG data files, which can be transferred onto the MUSE. All ECG files so acquired will then be analysed and over-read using the same process as for any other 12-lead ECG.

The system will be managed according to local working practices. The ECG telemetry reports will be archived with study documents.

12.10.4 Analysing and over-reading 12-lead ECG for the purpose of intensive cardiac assessments

Each electronic ECG will contain the ECG data as well as the result of the automated ECG analysis performed by the Marquette® 12SL™ ECG Analysis Program (MEAP), a program resident in each of the ECG machines. All ECG and their associated automated interval measurements will subsequently be reviewed by qualified Cardiologists in accordance with the ICH E14 Guidance for Industry document and ICH E14 Implementation Working Group Questions and Answers document before any of the ECG are used for the thorough ECG analysis. The manual adjudication process applied in this study is also referred to in the ICH guidance and relevant literature as “manual over-read”, “computer-assisted” or “semi-automated” ECG measurements. The following parameters on each ECG (but not limited to) will be assessed by a cardiologist using the commercially available MUSE® in its latest version:

- QT interval
- RR interval
- Heart rate (HR)
- PR interval
- Presence or absence of U-wave
- Quantitative and qualitative ECG variations

Manual on-screen over-reading using electronic callipers in MUSE® will be performed by a small and select group of cardiologists with extensive experience with manual QT measurement (including on-screen measurement with electronic callipers). For all study ECG the over-reading cardiologists will be blinded to time, date, treatment and any data identifying the subject. All ECG of a given subject will be over-read by the same cardiologist (or cardiologists in case manual adjustments of the automated measurement are necessary).

12.11 Pharmacokinetic Assessments

For timing of individual samples refer to the Study Plans (Table 2, Table 3, Table 4, and Table 5). The date and time of collection will be recorded on the appropriate CRF.

12.11.1 PK blood samples

Venous blood samples for the determination of concentrations of P218 and metabolite in plasma will be taken at the times presented in the Study Flow Charts (Table 2, Table 3, Table 4, and Table 5). For blood volume see Section 12.2. Samples will be collected, stored and shipped as detailed in a separate SOM.

All sample handling procedures, including the time of each sample collection, the time of placement into frozen storage (at the end of the sample workup), and the date of transfer or shipment of the samples to the responsible analyst will be documented in detail in the SOM. The procedures and materials used, e.g. collection and storage tubes, will be examined prior to any analytical measurements as part of the analytical method validation, to rule out any possible interference with the analyte.

Plasma samples for determination of P218 and metabolite concentration will be analysed by Swiss BioQuant laboratory on behalf of MMV using a validated HPLC/MS/MS method. Full details of the analytical methods used will be described in a separate bioanalytical report.

12.11.2 PK urine samples

Urine samples for determination of concentration of P218 will be taken from the total urine sample provided during each collection period presented in the Study Plans (Table 2, Table 3, Table 4, and Table 5). Samples will be collected, stored and shipped as detailed in a separate SOM.

Urine samples for determination of P218 and metabolite concentration will be analysed by Swiss BioQuant laboratory on behalf of MMV using a non-validated method. Should more than 10 % compound be found in the urine then a validated method will be used. Full details of the analytical methods used will be described in a separate bioanalytical report.

12.11.3 Serum and Red Blood Cell folate levels

Venous blood samples for the determination of the effect of P218 on serum and red blood cell folate levels will be taken at the times presented in the Study Plans (Table 2 and Table 4). For blood volume see Section12.2.

All sample handling procedures, and the date of transfer or shipment of the samples to the responsible analyst will be documented in detail in the SOM. The procedures and materials used, e.g. collection and storage tubes, will be examined prior to any analytical measurements as part of the analytical method validation, to rule out any possible interference with the analyte.

This procedure will be further detailed in the SOM.

12.11.4 Malaria bioassay

Venous blood samples taken to confirm that P218 β acyl glucuronide is active against Plasmodium parasite and to determine if other active metabolites are formed will be taken at the times presented in the Study Plans (Table 2, Table 3, Table 4 and Table 5). For blood volume see section 12.12. These samples will be analysed by Swiss TPH laboratory on behalf of MMV.

All sample handling procedures, and the date of transfer or shipment of the samples to the responsible analyst will be documented in detail in the SOM. The procedures and materials used, e.g. collection and storage tubes, will be examined prior to any analytical measurements as part of the analytical method validation, to rule out any possible interference with the analyte.

This procedure will be further detailed in the SOM.

12.11.5 Pharmacogenetics

This study includes an optional, exploratory pharmacogenetic component.

This blood sample (10-15 mL) should be collected prior to drug administration. Only one blood sample per subject will be collected.

Samples will be collected, stored and shipped as detailed in a separate SOM.

All sample handling procedures, including the time of each sample collection, the time of placement into frozen storage (at the end of the sample workup), and the date of transfer or shipment of the samples to the responsible analyst will be documented in detail in the SOM.

12.12 Volume of Blood Sampling

The maximum total blood volume collected from subjects participating in this study will not exceed 600 mL. Details will be described in the SOM. The total volume of blood will be obtained in small volumes over an extended time period and is not anticipated to propose any risk to the subjects.

13. ADVERSE EVENTS

The collection, evaluation and reporting of adverse events/reactions arising from this clinical study will be performed in accordance with:

- "Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3') (2011/C 172/01).
- International Conference on Harmonization (ICH) guideline E2F "Note for guidance on development safety update reports (DSUR)".

13.1 Adverse Events

It is the Investigator's responsibility to document and report all adverse events occurring in the clinical trial. The period of observation for collection of adverse events extends from the screening visit up to the final visit (including any wash-out or run-in periods).

All adverse events which come to the attention of the Investigator within 4 weeks from the end of the study treatment must also be recorded.

13.1.1 Definitions

According to ICH Topic E6, an **adverse event (AE)** is any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to it.

An **adverse reaction (ADR)** is a response to a medicinal product which is noxious and unintended and which occurs at any dose (in pre-approval clinical experience) or at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for modification of physiological function (in post-approval clinical experience). The term "response" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility. This means that there are facts (evidence) or arguments to suggest a causal relationship.

According to ICH Topic E9 a **treatment emergent adverse event/adverse reaction** is an event that emerges during treatment, having been absent pre-treatment, or worsens relative to the pre-treatment state.

In a clinical trial, in addition to the above mentioned "treatment-emergent" AEs/ADRs, any unfavourable and unintended sign, symptom or disease occurring in the study during any wash-out periods must also be recorded as an AE/ADR.

An AE/ADR may be:

- a new symptom or medical condition;

- a new diagnosis;
- an inter-current illness or an accident;
- a worsening of a medical condition/diseases existing before the start of the clinical trial;
- the recurrence of a disease;
- an increase in frequency or intensity of episodic diseases;
- a change in a laboratory parameter.

The criteria for determining whether an abnormal test result should be reported as an AE/ADR are as follows:

- the test result is associated with accompanying symptoms, and/or
- it requires additional diagnostic testing or medical/surgical intervention, and/or
- it leads to a change in trial dosing outside of protocol-stipulated dose adjustments, or discontinuation from the trial, significant additional concomitant drug treatment, or other therapy, and/or
- it is considered to be an adverse event by the Investigator or Sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE/ADR. Any abnormal test result that is determined to be an error does not require reporting as an adverse event.

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE/ADR. Planned surgical measures permitted by the clinical trial protocol and the condition(s) leading to these measures are not AEs, if the condition leading to the measure was present before inclusion in the trial. In the latter case the condition should be reported as medical history.

13.1.2 Classification

Seriousness

An adverse event/reaction is defined as serious if:

- it results in death;
- it is life-threatening (the term "life-threatening" in the definition of "serious" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe);
- it requires an hospitalisation or prolongs existing hospitalisation;
- it results in persistent or significant disability/incapacity;
- it is a congenital abnormality/birth defect;
- it is considered medically important (medical and scientific judgement should be exercised in deciding whether other AE/ADRs are to be considered serious, such as important medical events that may not be immediately life-threatening but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias; convulsions that do not result in hospitalisation; development of drug dependency or drug abuse).

A **non-serious** AE/ADR is any symptom or sign which does not fulfil any of the above-mentioned seriousness criteria.

A **Suspected Unexpected Serious Adverse Reactions (SUSAR)** is any SAE where a causal relationship with the IMP is at least a reasonable possibility, but the event is not listed in the Investigator Brochure and/or Summary of Product Characteristics.

For the purpose of this study, the following **adverse events of special interest (AESIs)** are defined:

Hepatic

- possible Hy's law case: defined as a subject with any value of ALT or AST above 3x ULN together with an increase in bilirubin to a value higher than 2x ULN and not associated with an ALP value higher than 2x ULN (to be reported as an SAE as detailed above)
- any ALT or AST above 3x ULN
- any elevation in bilirubin 2x ULN
- any AST or ALT above 2x ULN and (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

- QTcF prolongation from baseline (ECG within 1 hour prior to IMP administration) of >60 msec
- QTcF at any time >480 msec
- bundle branch block
- any arrhythmia.

Haematological

- Hb drop >2 g/dL from baseline (considered Day -1 for Part A, or pre-dose on Day D0 for Part B)
- Absolute neutrophil count <1000 /µL.

Cutaneous

- Dermatitis
- Rash
- Rash erythematous
- Rash macular
- Rash papular
- Rash maculo-papular
- Rash pruritic
- Rash pustular
- Rash vesicular

Gastrointestinal

- Nausea
- Vomiting
- Anorexia
- Constipation
- Stomatitis/Pharyngitis
- Diarrhea
- Dyspepsia/Heartburn

Although not considered an adverse event, pregnancy in the partner of a male subject will be reported as an **event of special interest**.

Expectedness

An adverse reaction, the nature or severity of which is not consistent with the applicable reference safety information (IB for drugs in clinical development or SmPC for marketed drugs) is unexpected. Reports which add significant information on the specificity, increase in the occurrence or severity of a known and already documented serious adverse reaction are unexpected events. The expectedness of an adverse reaction is determined by the Sponsor in the reference safety information. This should be done from the perspective of events previously observed, not on the basis of what might be anticipated from the pharmacological properties of a medicinal product.

For this protocol, the reference document for the assessment of expectedness is the Investigator's Drug Brochure for P218; however no clinical data are so far available for this compound.

Intensity

Please refer to section 8.3

Standard toxicity grading

Please refer to section 8.3

Causality assessment

The following criteria, based on WHO definitions, are used for causality assessment:

Definite:

- it has a reasonable time relationship to drug administration, and
- it cannot be explained by other factors (concurrent diseases or medications), and
- it regresses upon withdrawal of the drug, and
- it clearly follows a known pattern of response and it reappears after restart in case of re-challenge.

Probable:

- it has a reasonable time relationship to drug administration, and
- it cannot be reasonably explained by other factors (concurrent diseases or medications), and
- it regresses upon withdrawal of the drug.

Possible:

- it has a reasonable time relationship to drug administration, but
- it can be explained by other factors (concurrent diseases or medications).

Not-related:

- it has not a reasonable time relationship to drug administration or there is a time relationship, but the event does not follow a known pattern of response and,
- it is due to other factors

Treatment-emergent adverse events with a definite or probable or possible relationship to the medicinal product are to be considered as drug-related, i.e. adverse reactions.

The Investigator should also comment on the adverse event page of the Case Report Form whether an adverse event is not related to the study treatment, but it is related to the study participation of the subject (study procedures, wash-out periods etc).

13.1.3 Recording of adverse events and follow-up

All (serious and non-serious) adverse events detected by the Investigator or delegates, or spontaneously notified by the subject at each visit/examination must be reported on the special section of the CRF.

The following information should be reported for each adverse event, whether or not it can be attributed to trial drug:

- description of adverse event
- date of onset/date of disappearance
- characteristics of the event (seriousness, intensity)
- actions taken (treatment required or dose adjustments must be reported in the CRF)
- outcome
- relationship with trial drug (causality assessment) and/or study participation

All adverse events must be documented and followed up until the event is either resolved or a satisfactory explanation is found, or the investigator considers it medically justifiable to terminate the follow-up.

Spontaneously reported SAEs will be collected until 30 days following the final study visit.

SAEs experienced after this 30-day period will only be reported if the investigator suspects a causal relationship with the study drug.

13.1.4 Reporting of serious adverse events

If any SAE/SUSAR occurs, the investigators will take appropriate action immediately and will strive to identify the causes of the events.

Any SAE/SUSAR will be notified by the Investigator to the Sponsor within 24 hours by email or fax, using the MMV “Serious Adverse Event Report Form for Clinical Trials”, to;

<u>MMV Medical Monitor:</u>

Stephan Chalon
Email: chalons@mmv.org
Phone: +41 79 962 9244
Fax: +41 22 555 0369

Project Manager:
Susanne Riester
Operations Manager
Quintiles AG
Hochstr. 50
CH-4053 Basel
Switzerland
Office: + 41 61 270 81 81
Direct: + 41 61 270 81 66
Fax: + 41 61 270 81 80
susanne.riester@quintiles.com

The initial report will be followed up by a full written report within three working days or five calendar days, whichever comes first unless no further information is available when the follow-up report will be provided as soon as possible when new information becomes available. Further follow-up reports will be provided as and when new information becomes available. Photocopies of results, consultant report(s), a summary of the outcome of the reaction and the Investigator's opinion of IMP relationship to the SAE/SUSAR will accompany the SAE form if and when available.

The Sponsor will also perform an evaluation of the seriousness, causality and expectedness of all SAEs. All SAEs judged by either the Investigator or the Sponsor as having a reasonable suspected causal relationship to an IMP (i.e. definitively, probably or possibly related) will qualify as serious adverse reactions. If the Sponsor disagrees with the Investigator's causality assessment, both the opinion of the Investigator and the Sponsor are provided with the report.

All SAEs will be included in the MMV pharmacovigilance database.

SUSARs will be notified to the Competent Authority by MMV and to the relevant REC within 7 (for fatal and life-threatening SUSARs) or 15 days (all other SUSARs).

Annual safety reporting to the national Competent Authority and the Ethics Committee will be in agreement with ICH guideline E2F "Note for guidance on development safety update reports (DSUR)".

In addition, any other safety issue which may alter the current benefit–risk assessment of the IMP will be reported by the Sponsor (or delegate) on an expedited basis to Health Authorities, Ethics Committees and the Investigator.

The detailed procedure of the SAE/SUSAR reporting will be described in a Safety Management Plan that will be finalized before the start of the study to exactly define the different tasks of the Investigator, the Sponsor and RPL (on behalf of the Sponsor).

14. QUALITY ASSURANCE AND QUALITY CONTROL

14.1 Quality Assurance and Quality Control

A regulatory inspection of this study may be carried out by regulatory agencies. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the PI and RPL agree to allow the auditor/inspector direct access to all relevant documents and to allocate their time and the time of their staff to the auditor/inspector to discuss any findings or relevant issues.

Quality Control (QC) procedures at the CPU will be implemented to ensure data recorded into the CRFs are accurate before CRFs are sent for data entry purposes. QC checks will be carried out on critical phases in the execution of the study. These control checks will be carried out according to the relevant SOPs. Records of these procedures will be documented and available for review.

14.2 Monitoring

All aspects of the study will be carefully monitored by the sponsor, or designee, for compliance with applicable government regulations with respect to Good Clinical Practice (GCP) and current standard operating procedures.

The monitoring of this study will be performed by the Sponsor's Monitor(s) or a designee in accordance with the principles of GCP as laid out in the International Conference on Harmonisation (ICH) "Good Clinical Practice: Consolidated Guideline".

The clinical monitor, as a representative of the Sponsor, has an obligation to follow the study closely. In doing so, the monitor will visit the Investigator and site periodically as well as maintain frequent telephone and letter contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and staff. Further details will be described in the SOM.

15. STATISTICAL ANALYSIS

15.1 Statistical Analysis Plan

A statistical analysis plan (SAP) containing detailed statistical methodology will be written and signed off before the unblinding of the study. The plan will be updated to reflect adaptive features of the study as appropriate.

Because the nature of this study is primarily exploratory, the p-values from hypothesis tests and the confidence intervals (CIs) obtained in the statistical analyses are not intended to make inferences but to identify potential patterns and quantify the uncertainty of estimation.

15.2 Analysis Sets

The analysis of data will be based on different analysis sets according to the purpose of analysis. Subject eligibility for each analysis set will be finalised before unblinding of the data. A subject who withdraws prior to the last planned observation in a study period will be included in the analyses up to the time of discontinuation.

All subjects that were enrolled and received a dose of the study drug will be included in the data analysis. Subjects will be analysed as treated (i.e. in accordance with the study drug received, even if different from the one to which they were randomised).

Specific analysis populations (safety, PK and PD populations) will be defined in the SAP.

15.3 Analysis and Presentation of Data

Continuous data will be summarised using descriptive statistics (mean, median, standard deviation, minimum, maximum). Categorical data will be presented using N and % (using the number of subjects without missing data in the calculation).

In all treatment and dose group comparisons, data from all subjects who received placebo (Part A) will be pooled to form one placebo comparison group.

15.4 Subject Demographics and Baseline Characteristics (Parts A and B)

The subject disposition will be summarised. Study completion, study withdrawals and inclusion in analysis sets will be summarised and the reasons for withdrawal, exclusions from analysis sets and protocol violations will be listed.

Data for demographic variables will be listed by treatment, dose and subject. Descriptive statistics will be provided by treatment and dose.

Medical history, current medical conditions, results of laboratory screening tests, drug tests and any other relevant baseline information will be listed by treatment, dose and subject.

Previous and concomitant medication taken at any stage from 4 weeks prior to dosing and during the study period will be listed per treatment and dose level.

Other baseline characteristics will be listed only.

15.5 Statistical analysis of safety

Adverse events (AE) data will be listed and summarised . The number (and %) of subjects who had any AEs will be summarised for each dose and fasted state. All AEs will be listed by using system organ class (SOC) and preferred term (PT) assigned to the event using Medical Dictionary for Regulatory Activities (MedDRA). Furthermore, subjects who had an AE will be summarised by maximum severity of the AE(s). The number of subjects who had drug-related AEs will also be summarised. Any serious adverse events (SAEs) and/ or adverse events that led to withdrawal will be listed.

Vital signs data (SBP, DBP, HR, Temperature) will be listed and summarised, along with changes from baseline, using descriptive statistics. Out-of-reference-range values and postural drops (SBP>20mmHg, DBP>10mmHg) will be flagged.

All safety clinical laboratory data will be listed. Laboratory test results will also be compared to laboratory reference ranges and those values outside of the applicable range will be flagged as high (H) or low (L). The quantitative laboratory data, along with changes from baseline will be summarised using descriptive statistics. Change from baseline values at each assessment will be calculated as the assessment value minus the baseline value. The qualitative urinalysis data will be listed only.

Physical Examination data will be listed.

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 using descriptive statistics.

Furthermore, categorical analysis of QTcF data will be presented as follows:

- Absolute QTcF interval prolongation
    - QTcF interval > 450 ms
    - QTcF interval > 480 ms
    - QTcF interval > 500 ms
- Change from baseline in QTcF interval
    - QTcF interval increases from baseline > 30 ms
    - QTcF interval increases from baseline > 60 ms

15.6 Pharmacokinetics

The following PK parameters will be determined using non-compartmental method(s) from plasma concentration-time data : AUC_{last}, AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$, MRT, CL/F (for parent only), Vz/F (for parent only) and metabolites ratio.

Serum concentrations will be listed and summarised by time point. The PK parameters will be listed for each subject and summarized for each treatment group using descriptive statistics (N - the number of subjects, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum).

AUC_{inf} and C_{max} (dose-normalized and/or transformed, when appropriate) of P218 will be compared among the fasting dose-groups with dose as a fixed effect.

A preliminary assessment of dose proportionality for maximum observed concentration (C_{max}) and AUC will be conducted using a power model. In addition to visual inspection techniques a lack-of-fit test will be used to determine the validity of the power model.

A 90% CI will be constructed on the ratio of Cmax fed to Cmax fasting and a similar one will be constructed for AUC to obtain a preliminary estimate of the food effect of P218 on transformed parameters with fasting/fed treatment, period and sequence as fixed effects and subject as a random effect.

15.7 Exploratory

15.7.1 Pharmacodynamics

Serum folate levels

Serum folate levels and changes from baseline will be listed and summarised using descriptive statistics per treatment and time point.The figures of mean differences from baseline for each treatment versus time point on linear scale will be plotted.

Ex-vivo malaria assay

Bioassay analysis results will be listed and summarised together with changes from baseline, and individual subject plots of the results over time presented.

ECG analysis

ECG analysis may be performed and will comprise of adjudicated ECG triplicates selected from each timepoint which will be compliant with the correct recording and manual adjudication of ECGs in thorough QT/QTc studies. QTcF will be used for ECG analyses, unless there is a substantial heart rate effect, in which case the correction used for QTc (the most appropriate heart rate correction) will be calculated under blinded conditions by the statisticians. Details of the analysis will be given in the statistical analysis plan.

15.7.2 Pharmacogenetics

Pharmacogenetics samples will be stored for future optional analysis.

15.8 Handling of Missing and Incomplete Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) for handling missing data may be reassessed at the data review prior to database lock.

15.9 Determination of sample size

15.9.1 Sample Size Calculation

Because the nature of this study is primarily exploratory, the p-values from hypothesis tests and the confidence intervals obtained in the statistical analyses are not intended to make inferences but to identify the potential patterns and quantify the uncertainty of estimation. The following factors were taken into consideration when determining the sample size:

15.9.2 Part A (SAD):

Because the primary objective is an initial assessment of safety, each treatment group is limited to 6 subjects receiving P218. Administration of P218 to 6 subjects in each dose group provides a 47%, 62%, 74%, or 82% probability of observing at least 1 occurrence of any AE with a true incidence rate for a given dose group of 10%, 15%, 20%, or 25%, respectively.

Furthermore, it is assumed that pooling the data for the 2 placebo subjects who received placebo from each cohort will provide an adequately sized control group.

15.9.3 Part B (Food effect):

This is a pilot evaluation designed to determine whether P218 PK is impacted by food. Historically 8 participants in a dose cohort have proven sufficient to characterize the preliminary effects of food on safety and PK of a new chemical entity in healthy subjects. Therefore, the

size of the current cohort (n=8) is considered adequate at this stage of the drug development process.

16. DATA MANAGEMENT

Data Management will be performed by the Data Management department of RPL. The data management process will be described in detail in the Data Handling Protocol (DHP).

The RPL Data Management department will be responsible for developing and maintaining the DHP; setting-up and validating the clinical study database; programming validation checks; entering data into the clinical study database; reviewing data for accuracy, completeness and consistency between the CRF and the database; and verifying adherence to the clinical pharmacology study protocol and the DHP.

The study database will be built using Oracle Clinical based on the CRF design. Data Entry will be performed using double independent data entry method with second pass verification.

Safety laboratory data will be loaded into the database as an electronic data transfer file according to the Data transfer specification document.

Clinical data queries will be generated and resolved according to the DHP. They are documented individually on a Data Clarification Form (DCF) which is generated in Oracle Clinical. The DCF is a form designed to maintain an audit trail of modifications of the data in the clinical study database and the justification for those modifications. Clinical data queries are resolved with the assistance of RPL clinical staff.

After all clinical data queries are resolved, final error rate is confirmed and QC checks are acceptable the database will be locked.

Standard SAS® datasets are generated from the final study database ready for analyses. A complete audit trail of all corrections will be available for inspection. Medical coding will be performed by RPL.

AEs, diagnoses from Medical History and procedures from Surgical History will be classified according to MedDRA. Concomitant medication will be coded using WHODRUG.

SAEs in the clinical database will be reconciled with the safety database.

Final raw SAS® datasets will be transferred to statistician and sponsor (as applicable) according to the Data Structures Document (DSD).

16.1 Case Report Forms

Case Report Forms will be used to record the data in the study. Data should be recorded legibly onto the CRFs in black ballpoint pen. Correction fluid or covering labels must not be used.

The monitor will check data at the monitoring visits to the study site. The PI will ensure that the data in the CRFs are accurate, complete, and legible.

Data from the completed CRFs will be entered into RPL's clinical study database and validated under the direction of the Data Manager. Screening failures (subjects who signed consent to take part in the study but were not randomised) as well as admission data for Reserves will not be entered into the clinical study database. Any missing, impossible (inconsistent with human life), or inconsistent recordings in the CRFs will be referred back to the PI using a DCF and be documented for each individual subject before clean file status is declared.

17. SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

17.1 Sponsor's Responsibilities

17.1.1 GCP compliance

MMV and any third party to whom aspects of the study management or monitoring have been delegated will undertake their roles for this study in compliance with all applicable regulations and ICH GCP Guidelines.

Visits to Investigator sites will be conducted by representatives of MMV to inspect study data, subjects' medical records, and CRFs in accordance with current GCP and the respective local and national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by competent authorities.

17.1.2 Regulatory approval

MMV will ensure that Local Competent Authority requirements are met before the start of the study.

17.1.3 Indemnity/liability and insurance

MMV will adhere to the recommendations of the Association of British Pharmaceutical Industry (ABPI) Guidelines. A copy of the Indemnity document will be supplied to the Investigator before study initiation.

MMV will ensure that suitable insurance cover is in place prior to the start of the study. An insurance certificate and a statement of insurance will be supplied to RPL.

17.1.4 Protocol management

All protocols and amendments will be prepared by MMV and/or RPL. If it becomes necessary to issue a protocol amendment during the course of the study, MMV will notify the Investigator and collect documented Investigator Agreement to the amendment.

17.1.5 End of trial notification

MMV will submit an end of trial notification to the competent authority of the Member State within 90 days of the end of the trial in accordance with EU Directive 2001/20/EC. The PI will be responsible for submitting these to the REC within 90 days of the end of the trial.

For the purposes of this notification, the end of the trial will be defined as the last subject/last visit.

17.1.6 Submission of summary of clinical trial report to competent authorities of member states concerned and RECs Regulatory approval

MMV will provide a summary of the clinical trial report within one year of the end of the complete trial to the competent authority of the Member State concerned as required by the regulatory requirement and to comply with the Community guideline on Good Clinical Practice.

17.2 Investigator's Responsibilities

17.2.1 GCP compliance

The Investigator must undertake to perform the study in accordance with ICH GCP Guidelines, EU Directive 2001/20/EC, and the applicable regulatory requirements.

It is the Investigator's responsibility to ensure that adequate time and appropriate resources are available at the study site prior to commitment to participate in this study. The Investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The Investigator will maintain a record of appropriately qualified persons to whom the Investigator has delegated significant trial-related tasks. An up-to-date copy of the *curriculum vitae* for the Investigator, Sub-investigator(s), and essential study staff will be provided to MMV (or designee) before starting the study.

Agreement with the final Clinical Study Report will be documented by the dated signature of the PI, in compliance with Directive 75/318/EC, Directive 2001/83/EC, and ICH E3.

17.2.2 Protocol adherence and investigator agreement

The PI and delegates must adhere to the CSP as detailed in this document. The PI will be responsible for enrolling only those subjects who have met CSP eligibility criteria. The PI will be required to sign an Investigator Agreement to confirm acceptance and willingness for themselves and delegates to comply with the CSP.

17.2.3 Documentation and retention of records

After completion of the study, all documents and data relating to the study will be kept in an orderly manner and securely by the PI in a secure file and/or electronically. The data will be available for inspection by MMV or their representatives. Essential documents must be retained for 2 years after the final marketing approval in an ICH region or at least 2 years have elapsed since the discontinuation of clinical development of P218. The PI or delegate must contact MMV before destroying any study-related documentation and it is the responsibility of MMV to inform the investigative site of when these documents can be destroyed. In addition, all subject records and other source documentation will be kept for a longer period if required by the applicable regulatory requirements.

17.3 Ethical Considerations

This protocol complies with the principles of the World Medical Assembly (Helsinki 1964) and subsequent amendments.

17.3.1 Informed consent

The informed consent is a process by which a subject voluntarily confirms his/her willingness to participate in a clinical study. It is the responsibility of the PI or delegate to obtain written informed consent from subjects. All consent documentation must be in accordance with applicable regulations and GCP. Each subject is requested to sign the Informed Consent Form (ICF) after they have received and read the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. Signed ICFs

must remain on file and must be available for verification by Study Monitors at any time. Another signed original of the ICF must be given to the subject or the subject's legally authorized representative. The PI or delegate will provide the Sponsor with a copy of the REC approved consent forms, and a copy of the REC written approval, prior to the start of the study.

17.3.2 Research Ethics Committee (REC) approval

It is the responsibility of the PI to submit this CSP, the informed consent document (approved by MMV), relevant supporting information, and all types of subject recruitment information to the REC for review, and all must be approved prior to the start of subject screening. In addition, advertisements must be approved by the REC prior to use at the site. Prior to implementing changes in the study, MMV and the REC must also approve any substantial amendments to the CSP and corresponding updates to informed consent documents. For non-substantial protocol amendments (that do not require REC approval) and subsequent updates of the ICF all changes will be done in agreement with MMV and RPL.

17.4 Confidentiality

Data collected during this study may be used to support the development, registration, or marketing of medicinal product. MMV will control all data collected during the study, and will abide by the EU Directive on Data Privacy concerning the processing and use of subjects' personal data. For the purpose of data privacy legislation, MMV will be the data controller.

After subjects have consented to take part in the study, their medical records and the data collected during the study will be reviewed by MMV and/or its representatives. These records and data may, in addition, be reviewed by the following: independent auditors who validate the data on behalf of MMV; national or local regulatory authorities, and the REC which gave its approval for this study to proceed.

Although subjects will be known by a unique number, their initials and date of birth will also be collected and used to assist MMV to verify the accuracy of the data, for example, that the results of study assessments are assigned to the correct subject. The results of this study containing the unique number, initials, date of birth, and relevant medical information including ethnicity may be recorded and transferred to and used in other countries throughout the world, which may not afford the same level of protection that applies within the EU. The purpose of any such transfer would be to support regulatory submissions made by MMV in such countries.

17.5 Publication Policy

If the Sponsor and RPL agree that it will be desirable to publish the results of this study; both parties will liaise in good faith to publish the results, RPL agree to obtain the Sponsor's prior written approval of such publications.

18. REFERENCES

Ferber G, Wang D, Täubel J. Concentration-effect modelling based on change from baseline to assess the prolonging effect of drugs on QTc together with an estimate of the circadian time course. *J Clin Pharmacol* 2014, doi: 10.1002/jcph.3478.

World Malaria Report 2014. World Health Organization. Geneva, Switzerland (http://www.who.int/publications/en/). Accessed January 2015.

Yuthavong Y, Tarnchompooa, B, Vilaivanb T *et al*. Malarial dihydrofolate reductase as a paradigm for drug development against a resistance-compromised target. PNAS 2012; 109(42): 16823-16828.

19. APPENDIX LIST

APPENDIX A – WHO Toxicity Grading Scale for determining severity of adverse events

ABBREVIATIONS (used in the table)

ULN=Upper Limit of Normal
LLN=Lower Limit of Normal
R_x = Therapy
IV = Intravenous
ADL = Activities of Daily Living

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

GRADE 1	**Mild**	Transient or mild discomfort (<48 hours); no medical intervention/therapy required.
GRADE 2	**Moderate**	Mild to moderate limitation in activity; some assistance may be needed; no or minimal medical intervention/ therapy required.
GRADE 3	**Severe**	Marked limitation in activity; some assistance usually required; medical intervention/therapy required; hospitalizations possible.
GRADE 4	**Potentially life threatening** [a]	Extreme limitation in activity; significant assistance required; significant medical intervention/therapy required; hospitalization or hospice care probable

[a] Revised by the Sponsor

COMMENTS REGARDING THE USE OF THIS TABLE

For parameters not included in the following Toxicity Tables, sites should refer to the "Guide For Estimating Severity Grade" located above.

Criteria are generally grouped by body system.

Item	Grade 1	Grade 2	Grade 3	Grade 4
Hematology				
Hemoglobin	9.5-10.5 gm/dL	8.0-9.4 gm/dL	6.5-7.9 gm/dL	<6.5 gm/dL
Absolute Neutrophil Count	1000-1500/mm³	750-999/mm³	500-749/mm³	<500/mm³
Platelets	75000-99000/mm³	50000-74999/mm³	20000-49999/mm³	<20000/mm³
Prothrombin Time (PT)	≥1.01 to ≤1.25 x ULN	>1.25 to ≤1.50 x ULN	>1.50 to ≤3.00 x ULN	>3.00 x ULN
Activated Partial Thromboplastin (APPT)	≥1.01 to ≤1.66 x ULN	>1.66 to ≤2.33 x ULN	>2.33 to ≤3.00 x ULN	>3.00 x ULN
Fibrinogen	≥0.75 to ≤0.99 x LLN	≥0.50 to <0.75 x LLN	≥0.25 to <0.50 x LLN	<0.25 x LLN
Fibrin Split Product	20-40 mcg/mL	41-50 mcg/mL	51-60 mcg/mL	>60 mcg/mL
Methemoglobin	5.0-9.9%	10.0-14.9%	15.0-19.9%	>20.0%
Liver Enzymes				
AST (SGOT)	≥1.25 to ≤2.50 x ULN	>2.50 to ≤5.00 x ULN	>5.00 to ≤10.00 x ULN	>10.00 x ULN
ALT (SGPT)	≥1.25 to ≤2.50 x ULN	>2.50 to ≤5.00 x ULN	>5.00 to ≤10.00 x ULN	>10.00 x ULN
GGT	≥1.25 to ≤2.50 x ULN	>2.50 to ≤5.00 x ULN	>5.00 to ≤10.00 x ULN	>10.00 x ULN
Alkaline Phosphatase	≥1.25 to ≤2.50 x ULN	>2.50 to ≤5.00 x ULN	>5.00 to ≤10.00 x ULN	>10.00 x ULN
Amylase	≥1.1 to ≤1.5 x ULN	>1.5 to ≤2.0 x ULN	>2.0 to ≤5.0 x ULN	>5.0 x ULN
Chemistries				
Hyponatremia	130-135 mEq/L	123-129 mEq/L	116-122 mEq/L	<116 mEq/L or mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	158-165 mEq/L	>165 mEq/L or mental status changes or seizures
Hypokalemia	3.0-3.4 mEq/L	2.5-2.9 mEq/L	2.0-2.4 mEq/L or intensive replacement Rx required or hospitalization required	<2.0 mEq/L or paresis or ileus or life-threatening arrhythmia
Hyperkalemia	5.6-6.0 mEq/L	6.1-6.5 mEq/L	6.6-7.0 mEq/L	>7.0 mEq/L or life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or mental status changes or coma
Hyperglycemia (note if fasting)	116-160 mg/dL	161-250 mg/dL	251-500 mg/dL	>500 mg/dL or ketoacidosis or seizures

Item	Grade 1	Grade 2	Grade 3	Grade 4
Cough	Transient; no Rx	treatment associated cough local Rx	uncontrolled	-
Bronchospasm, Acute	transient; no Rx <80-70% FEV_1 (or peak flow)	requires Rx normalizes with bronchodilator; FEV_1 50-70% (or peak flow)	no normalization with bronchodilator; FEV_1 25-50% (or peak flow retractions)	cyanosis: FEV_1 <25% (or peak flow) or intubated
Stomatitis	mild discomfort; no limits on activity	some limits on eating/drinking	eating/talking very limited	requires IV fluids
Nausea	mild discomfort; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	severe discomfort; no significant intake; activities limited	minimal fluid intake
Vomiting	transient emesis	occasional/moderate vomiting	orthostatic hypotension or IV fluids required	hypotensive shock or hospitalization required for IV fluid therapy
Constipation	mild	moderate	severe	distensions w/vomiting
Diarrhea	transient 3-4 loose stools/day	5-7 loose stools/day	orthostatic hypotension or >7 loose stools/day or required IV fluids	hypotensive shock or hospitalization for IV fluid therapy required
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Mood	mild anxiety or depression	moderate anxiety or depression and therapy required	severe anxiety or depression or mania; needs assistance	acute psychosis; incapacitated, requires hospitalization
Neuro Control (ADL = activities of daily living)	mild difficulty concentrating; no Rx; mild confusion/agitation; ADL unaffected	moderate confusion/agitation some limitation of ADL; minimal Rx	severe confusion/agitation needs assistance for ADL; therapy required	toxic psychosis; hospitalization
Muscle Strength	subjective weakness no objective symptoms/ signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis

Item	Grade 1	Grade 2	Grade 3	Grade 4
Other Parameters				
Fever: oral, >12 hours	37.7-38.5 C or 100.0-101.5 F	38.6-39.5 C or 101.6-102.9 F	39.6-40.5 C or 103-105 F	>40 C or >105 F
Headache	mild, no Rx therapy	transient, moderate; Rx required	severe; responds to initial narcotic therapy	intractable; required repeated narcotic therapy
Fatigue	no decrease in ADL	normal activity decreased 25-50%	normal activity decreased >50% can't work	unable to care for self
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Local Reaction	tenderness or erythema	induration <10 cm or phlebitis or inflammation	induration >10 cm or ulceration	necrosis
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation, moist desquamation, or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery

For cholesterol and LDL cholesterol, the following severity gradings should be applied:

Cholesterol (fasting)

Adult ≥ 18 years

Grade 1: 200 – 239 mg/dL or *5.18 – 6.19 mmol/L*

Grade 2: 240 – 300 mg/dL or *6.20 – 7.77 mmol/L*

Grade 3: >300 mg/dL or *>7.77 mmol/L*

Grade 4: NA

LDL cholesterol (fasting)

Adult ≥ 18 years

Grade 1: 130 – 159 mg/dL or *3.37 – 4.12 mmol/L*

Grade 2: 160 – 190 mg/dL or *4.13 – 4.90 mmol/L*

Grade 3: ≥ 191 mg/dL or ≥ *4.91 mmol/L*

Grade 4: NA