STATISTICAL ANALYSIS PLAN

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

Protocol:	MMV_P218_17_01
SGS LS number:	BE-80-1803670
Development phase:	Ib
Sponsor:	Medicines for Malaria Venture (MMV)
SAP version number:	Final 1.0
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SIGNATURE PAGE

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PROTOCOL HISTORY

Protocol:		
Version or ID	**Date (ddMMMyyyy)**	**Impact of the changes on the statistical analysis**
Final	25SEP2018	NAP
Final 2.0	20MAR2019	Including substantial amendment 1: Dose of cohort 3 de-escalated from 1000 mg (i.e. cohort 2 dose) to 100 mg Days of administration in cohort 3 were changed from D3 and D5 to D1 and D3, which is as in cohort 2. Therefore, placebo subjects of cohort 3 can now be pooled with placebo subjects of cohort 2 in by time-point summaries as well. PK/PD relationship was added as a secondary objective and endpoint. A PK/PD population was added

This statistical analysis plan (SAP) only considers the latest version of the protocol, and of the protocol amendments, as listed above.

LIST OF ABBREVIATIONS

ADaM	analysis data model
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine transaminase
AST	aspartate transaminase
BLQ	below the limit of quantification
bpm	beats per minute
BUN	blood urea nitrogen
CHMI	controlled human malaria infection
CI	confidence interval
CK	creatinine kinase
CPK	creatine phosphokinase
CRF	case report form
CRP	C-reactive protein
CV	coefficient of variation
DBP	diastolic blood pressure
DVI	direct venous inoculation
DY	relative day
ECG	electrocardiogram
EOS	end-of-study
GGT	gamma-glutamyl transferase
GMR	geometric mean ratio
HR	heart rate
ICF	informed consent form
ICH	International Council for Harmonisation
IMP	investigational medicinal product
LDH	lactate dehydrogenase
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
NAP	not applicable

PD	pharmacodynamic(s)
P falciparum	*Plasmodium falciparum*
PfSPZ	*P. falciparum* sporozoites
PK	pharmacokinetic(s)
PP	per protocol
qPCR	quantitative polymerase chain reaction
QTc	corrected QT interval
QTcB	Bazett's corrected QT interval
QTcFri	Fridericia's corrected QT interval
RBC	red blood cell
RND	all randomised subjects analysis set
SAP	statistical analysis plan
SAF	safety analysis set
SBP	systolic blood pressure
SCR	all screened subjects analysis set
SD	standard deviation
SE	standard error
SGS LS	SGS Life Sciences
SOP	standard operating procedure
SRT	safety review team
STAT	statistics
TBS	thick blood smear
TEAE	treatment-emergent adverse event
TLF	tables, listings and figures
VS	vital signs
WBC	white blood cell
WHO	World Health Organisation
WI	work instruction

DEFINITION OF TERMS

baseline	An initial measurement of a condition that is taken at an early time point and used for comparison over time to look for changes. The study specific definition is in section 5.2.2.
case report form (CRF)	A printed, optical, or electronic document designed to record protocol required information to be reported to the sponsor for each trial subject.
display	Analysis table, figure or listing
phase	Interval of time in the planned conduct of a study associated with a specific purpose: for example, screening, treatment, follow-up.
round half up tie-breaking rule	Convention to round values ending with 5 to positive infinity. The round half up rule is implemented in the SAS® ROUND function.

Examples:

Database value	Rounded value
-1.35	-1.3
-1.25	-1.2
1.25	1.3
1.35	1.4

significant digit	All digits of a number used to express it to the required degree of accuracy, starting from the first non-zero digit.
study drug	Pharmaceutical form of an active ingredient or placebo, being tested or used as a reference in a clinical study.
treatment-emergent abnormality	Any post-baseline abnormality that was not present at baseline (e.g. haemoglobin normal at baseline and grade 1 post-baseline; glucose low at baseline and high post-baseline; QTcFri]450; 480] ms at baseline and >500 ms post-baseline)

TABLE OF CONTENTS

1. INTRODUCTION

This SAP describes the final statistical analysis to be performed for the MMV_P218_17_01 (BE-80-1803670) study.

This SAP covers the pharmacodynamic (PD), pharmacokinetic (PK), safety, and general characteristics parts of the statistical analysis. It specifies the analysis displays to be presented and describes the methods and procedures in a more elaborated way than in the statistical methods section of the protocol. The PK/PD analysis is not in scope of this SAP.

The statistical analysis will process and present the results following the International Council for Harmonisation (ICH) standards, in particular the ICH-E3, ICH-E6, and ICH-E9 guidelines.

1.1 STUDY OBJECTIVES

According to the protocol, the primary objective of cohort 1 is to assess the safety and tolerability of two single doses of 1000 mg P218 administered 48 hours apart in healthy adult volunteers. The primary objective of cohorts 2 and 3 is to assess the chemoprotective activity of P218 in *Plasmodium falciparum* (*P. falciparum*) controlled human malaria infection (CHMI) in non-immune healthy volunteers after *P. falciparum* sporozoites (PfSPZ) challenge through direct venous inoculation (DVI).

According to the protocol, secondary objectives are:

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

1.2 STUDY DESIGN

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

Subjects of cohort 1 are admitted to the clinical unit in the morning of Day -1 and are confined to the unit for 3 days thereafter (until Day 4, i.e. 36 hours after second

investigational medicinal product (IMP) administration), for close safety monitoring and PK assessments. First administration of 1000 mg of P218 or placebo takes place on Day 1. Second administration of 1000 mg of P218 or placebo takes place 48 hours later, on Day 3. After discharge from the clinical unit on Day 4, subjects are followed up with daily ambulatory visits to the clinical unit up to Day 9.

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

Subjects of Cohort 2 are admitted to the clinical unit in the morning of Day -1 and are confined to the unit until 12 days post the PfSPZ Challenge on Day 1 (i.e. until Day 13), for close safety monitoring and PK assessments. Each subject is administered 3200 *P. falciparum* sporozoites by DVI. First administration of 1000 mg of P218 or placebo takes place 2 hours after PfSPZ Challenge inoculation. Second administration of 1000 mg of P218 or placebo takes place 48 hours after first administration of P218 or placebo, on Day 3.

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

Subjects in Cohort 3 are admitted to the clinical unit in the morning of Day -1. On Day 1, subjects are administered 3200 *P. falciparum* sporozoites by DVI and are confined to the unit until 12 days post PfSPZ Challenge (i.e. until Day 13), for close safety monitoring and PK assessments. 100 mg of P218 or placebo are administered on Day 1 (2 hours after PfSPZ Challenge inoculation) and 48 hours after first administration of P218 or placebo, on Day 3.

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

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

The assessments performed are summarized per visit in the schedule of assessments in appendix 9.3.

1.3 EXPECTED SAMPLE SIZE

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

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

1.4 RANDOMISATION AND BLINDING

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

Subjects are assigned to 1 of 3 cohorts. Within each cohort, they are randomised in a 3:1 ratio to receive two consecutive administrations of either P218 or placebo. Allocation of each subject to a given treatment are described in a randomization list prepared prior to study start by SGS Life Sciences Secure Data Office using SAS® software.

The randomization is using randomly permuted blocks across the treatment groups.

In agreement with the Sponsor, additional subjects may be recruited in each cohort, to replace discontinuations for non-safety reasons and achieve cohort sizes of 8 (cohort 1) or 12 (cohorts 2 and 3). Replacement subjects receive the number of the subject to be replaced, increased by 100, and are administered the same treatment.

Blinding is achieved with placebo identical in appearance.

1.5 INTERIM ANALYSIS

No interim analyses are foreseen.

1.6 SOFTWARE

SAS version 9.4 or later will be used for programming.

WinNonlin Phoenix 8.0 or later (Pharsight Corporation, Palo Alto, Ca, USA) will be used for calculations of PK parameters.

1.7 VALIDATION MODEL

SGS Life Sciences (SGS LS) – Clinical Research statistics (STAT) standard operating procedures (SOPs) and work instructions (WIs) as effective at the project start will be followed throughout the project, in full compliance with the applicable regulatory requirements.

Primary endpoint tables will be validated according to model C, All other tables/figures/listings follow validation model B (see SOP.STAT.020):

- Model B: review by an independent person (biostatistician)
- Model C: review by an independent person and independent programming of tables 14.2.1.1 and 14.3.1.1

PK tables/figures/listings will be validated following model B (see SOP.PK.020 for more details):

- Model B: review by an independent person (pharmacokineticist)

PK analysis will be validated according SOP.PK.001.

2. PHARMACODYNAMIC AND PHARMACOKINETIC ANALYSES

2.1 PHARMACODYNAMICS

2.1.1 Available data

For the challenge cohorts 2 and 3, parasitaemia and malaria signs and symptoms will be assessed pre-challenge and daily from day 7 on.

Parasitaemia is assessed by qPCR and thick blood smear (TBS) microscopy.

The following malaria signs and symptoms will be scored: headache, myalgia, arthralgia, fatigue/lethargy, malaise, chills/shivering/rigors, sweating/hot spells, anorexia, nausea, vomiting, abdominal discomfort, fever, tachycardia and hypotension. Each of the previously mentioned symptoms is attributed a severity score that can be one of the following: 0 (absent), 1 (mild), 2 (moderate) and 3 (severe).

2.1.2 Derivation rules

The primary endpoint is time to positive parasitaemia, in days, which is calculated as: (date of first positive parasitaemia - date of inoculation + 1)/(24*60*60).

> Positive parasitaemia is defined as qPCR outcome (MBTESTCD = PARADENS) ≥ 250 asexual parasites per mL.
>
> In the absence of positive parasitaemia, the duration will be set to a maximum of 28 days. Consistently, for the Kaplan-Meier plot, subjects without positive parasitaemia will be censored at 28 days.

Malaria clinical score is calculated as the sum of all (14) malaria sign and symptoms scores (maximum score is 42). Missing individual scores will be imputed as detailed in section 5.3.1. The score will be rounded as detailed in section 5.3.4.

For individual signs and symptoms, a worst-case analysis visit, as defined in section 5.2.5 will be derived.

2.1.3 Presentation of results

The primary analysis is the summary of the time to positive parasitaemia by means of descriptive statistics, including the geometric mean and corresponding two-sided 90% confidence interval (CI).

The following secondary analyses will be performed:

- The incidence of positive parasitaemia between inoculation with PfSPZ and Day 28 will be presented, including two-sided 90% exact Clopper-Pearson CI.
- For the malaria clinical score, actual values and changes from baseline will be evaluated by means of descriptive statistics at each time point and at the worst-case analysis visit. This will be done by analysis phase (treatment + rescue / treatment).
- Individual malaria signs and symptoms scores will be presented as frequency tabulations at the worst-case analysis visit. Numbers and percentage of subjects with any post-baseline non-zero score will also be shown.

Graphs of the median actual values over time and spaghetti plots will be prepared for parasitaemia and malaria clinical score. For the spaghetti plots, the median will also be plotted and different plotting symbols will be used on days on which rescue medication was given. A Kaplan-Meier plot of the time to positive parasitaemia will also be shown.

Parasitaemia and malaria signs and symptoms will be listed, including all derived parameters. The onset date of rescue medication will be added to the listing.

2.2 PHARMACOKINETICS

2.2.1 Available data

Blood samples will be collected for the determination of P218 in all cohorts, and its major metabolites (P218 β-acyl-glucuronide, P218-OH β-acyl-glucuronide and P218-OH) in Cohort 1 only, at the time points indicated in the flow charts (see section 9.3). Allowed time deviations from the nominal time are:

- ± 5 min from 0.5 to 4 hours post dose;
- ± 15 min from >4 to 12 hours post dose;
- ± 30 min >12 to <24 hours post dose;
- ± 60 min for 24 hours post dose;
- ± 10% deviation from theoretical post dose times for >24 hours to <120 hours post dose;
- A deviation of ± 1 day for further days.

Predose sample should be taken with 1 hour before IMP administration.

The time zero is defined as the moment of the last IMP administration before the sampling. All concentration data-points with deviations outside these permitted ranges will be excluded from the descriptive statistics on concentrations, explained by a footnote in the appropriate tables, but kept in PK parameters estimation.

2.2.2 Derivation rules

Pharmacokinetic parameter calculations will be performed using Phoenix WinNonLin 8.0 or later (Pharsight Corporation, Palo Alto, CA, USA). The PK analysis will be based on actual times.

Standard noncompartmental methods will be used for the calculation of the following parameters from the individual plasma drug concentrations versus time profile, for P218, and in Cohort 1 only also for its major metabolites (P218 β-acyl-glucuronide, P218-OH β-acyl-glucuronide and P218-OH):

- AUC_{τ} = $AUC_{0\text{-}48h}$ (area under the plasma concentration-time curve from time zero until 48 h post-dose) after each administration (i.e. $AUC_{0\text{-}48h}$ and $AUC_{48\text{-}96h}$);
- $AUC_{48h\text{-}inf}$ (calculated from $AUC_{48h\text{-}t}$ + ($C_t/\lambda z$), where C_t is the last observed quantifiable concentration and λz the first order terminal rate constant), after the second administration;
- $AUC_{48h\text{-}last}$ (area under the plasma concentration-time curve from time zero until the last observed quantifiable concentration), after the second administration;
- CL/F (apparent oral clearance, calculated as Dose/$AUC_{48h\text{-}inf}$) and Vz/F (apparent volume of distribution during the terminal phase, calculated as Dose/(λz * $AUC_{48h\text{-}inf}$)) after the second administration (not for metabolites);
- C_{max} (maximum observed plasma concentration) and t_{max} (the time of occurrence of C_{max}) after each administration;
- $t_{1/2}$ (apparent terminal half-life, calculated from (ln 2)/λ_z) after the second administration;
- R_{ac}, calculated as $AUC_{48\text{-}96h}/AUC_{0\text{-}48h}$;
- Metabolic ratio of AUCτ and $AUC_{48h\text{-}inf}$ for P218 β-acyl glucuronide, P218-OH and P218-OH β-acyl glucuronide over parent P218 (Cohort 1 only), calculated as [(AUC for metabolite)*100/(sum of AUC for P218 and AUC of each of the 3 metabolites)].

Additional pharmacokinetic parameters may be calculated as appropriate.

For PK analysis, the following rules will be applied:

- Concentration below limit of quantification (BLQ) will be imputed according to the rules mentioned in section 5.3.3.
- Exposures (areas under the curve) will be calculated according to linear up/log down trapezoidal method using actual sampling time points
- Terminal elimination half-life estimation: At least three time points (excluding t_{max}) should be used for determination of the elimination rate constant (λ_z); the adjusted correlation coefficient of the linear regression should be ≥0.85. If these two criteria are not met the terminal elimination will not be reported as well as all derived parameters ($AUC_{48h\text{-}inf}$, CL/F and Vz/F).
- When for a subject the $AUC_{48h\text{-}last}$ / $AUC_{48h\text{-}inf}$ > 20%, this $AUC_{48h\text{-}inf}$, as well as all derived parameters (CL/F and Vz/F), will be excluded from the statistical analyses
- First administration (Day 1) predose values exceeding 5% of the C_{max} will be flagged in the tables.

Rules on how to deal with missing values when deriving PK AUC:

- If the first administration (Day 1) predose value is missing, then it will be set to 0.
- If intermediate values (not the predose, not more than 3 and not 2 consecutive) are missing, they will be considered as missing for the PK analysis.
- If multiple (>1) adjacent values are missing or more than 3 values are missing, then the AUCs will also be set to missing.

PK parameters that cannot be estimated will be reported as missing. More specific details will be provided in the PK Data Review document.

2.2.3 Presentation of results

All data issues with how the issue will be handled will be listed per subject, cohort, day of administration and time point (if applicable).

Actual blood sampling times from the last IMP administration for PK assessments will be listed.

Individual P218, P218 β-acyl-glucuronide, P218-OH β-acyl-glucuronide and P218-OH concentration data together with descriptive statistics will be presented in tables, per cohort and treatment, day of administration and time point. Individual data excluded from statistics will be flagged.

Individual P218, P218 β-acyl-glucuronide, P218-OH β-acyl-glucuronide and P218-OH PK parameters together with descriptive statistics will be presented in tables, per cohort and treatment, and day of administration. Individual data excluded from statistics will be flagged.

All plasma concentration/time curves for P218, P218 β-acyl-glucuronide, P218-OH β-acyl-glucuronide and P218-OH will be presented in linear/linear and log/linear scale. Mean (±SD) graphs will be presented as followed:

- with all treatments on the same graph, one graph for the first administration and another one for the second administration;
- with all treatments and all administrations on the same graph (from predose of the first administration to 192 h (9 days) post first administration).

@Sponsor: Please confirm whether this is what you expect

Spaghetti plots will be presented as followed:

- by treatment separately, for the two days of administration separately
- by treament separately, with both days of administration on the same graph (from predose of the first administration to 192 h (9 days) post first administration)

@Sponsor: Please confirm whether this is what you expect

Individual graphs will be presented per cohort and treatment, and subject, with both days of administration on the same graph.

3. SAFETY ANALYSES

3.1 ADVERSE EVENTS

3.1.1 Available data

Adverse events (AEs) are coded into system organ classes and preferred terms using the medical dictionary for regulatory activities (MedDRA). For each AE, start and stop date(time)s are collected as well as severity, a seriousness flag, P218 relatedness, action taken towards the study drug, outcome and a flag for AEs of special interest (listed in appendix 9.2). For cohorts 2 and 3, relatedness to PfSPZ inoculum, procedure and rescue drug will also be collected.

3.1.2 Derivation rules

Treatment-emergent adverse events (TEAE) are defined as AEs starting on or after first administration of any study drug. In case the AE start date(time) is incomplete or missing, AE will be considered as TEAE unless the available parts of the AE start or stop date(time) provide evidence for the event to be a pre-existing condition.

A fatal AE is defined as an AE with outcome ‘fatal’.

An AE for which the study was discontinued is defined as an AE mentioned as reason for study discontinuation. An AE for which the study drug was discontinued is defined as an AE with action taken ‘drug withdrawn’.

Relatedness will be dichotomised as follows in tables:

- Related: Related/suspected or missing
- Not related: Not related/not suspected or NAP

Treatment relatedness will be assessed for P218, and for challenge cohorts 2 and 3 also for PfSPZ inoculum, procedure and rescue drug.

AE onset and duration will be calculated as follows when start and stop dates are fully known:

- AE onset (days / hours) (vs. first dose) =
  - AE start date > reference date: AE start date – reference date + 1 day
  - AE start date < reference date: AE start date reference date
  - AE start date = reference date: (AE start datetime – reference datetime)/(60*60)

    The reference date is the date of first administration of any study drug.
- AE onset day (vs. start of phase) =
  - AE start date > analysis phase start date: AE start date – analysis phase start date + 1 day
  - AE start date = analysis phase start date: (AE start datetime – analysis phase start datetime)/(60*60)

- AE duration (days) =
  - Resolved AEs: AE end date – AE start date + 1 day
  - Unresolved AEs: study discontinuation date – AE start date + 1 day
    In this case the duration will be presented as ">x days".

3.1.3 Presentation of results

Tables will present TEAEs only. Pre-treatment AEs will only be listed. Unless stated otherwise, tables will not be shown by phase (treatment/rescue).

An overview table will show the number and percentage of subjects with at least one event and the number of events for the following:

- TEAEs
- Serious TEAEs
- Non-serious TEAEs
- Grade ≥ 3 TEAEs
- Fatal TEAEs
- P218 related TEAEs
- TEAEs related to PfSPZ inoculum (Cohorts 2 and 3 only),
- TEAEs related to procedure (Cohorts 2 and 3 only)
- TEAEs related to rescue drug (Cohorts 2 and 3 only)
- Serious P218 related TEAEs
- TEAEs for which the study was discontinued
- TEAEs for which the study drug was discontinued
- TEAEs of special interest

Summary tables by MedDRA system organ class and preferred term will show the number and percentage of subjects with at least one event. The table of TEAEs will additionally show the number of events. Separate tables will be prepared for the following:

- TEAEs (by phase (treatment/rescue - challenge cohorts only) and overall)
- Serious TEAEs
- Non-serious TEAEs *This table is needed for the legally required results posting to the EU clinical trials register (former EudraCT, newly renamed to EUCTR), so we would advise to keep it.*
- Grade ≥ 3 TEAEs
- P218 related TEAEs
- TEAEs related to PfSPZ inoculum (Cohorts 2 and 3 only),
- TEAEs related to procedure (Cohorts 2 and 3 only)
- TEAEs related to rescue drug (Cohorts 2 and 3 only)
- Serious P218 related TEAEs
- TEAEs of special interest

Additionally, a summary table by MedDRA preferred term will show the number and percentage of subjects with at least one event, by descending order of frequency (number of subjects with events in the active (total) group). This table will be shown by phase (treatment/rescue - challenge cohorts only) and overall.

All AEs, including pre-treatment events will be listed. Separate listings will be prepared for serious AEs (including the reason of seriousness), AEs after rescue medication, AEs for which the study and the study drug was discontinued and fatal AEs. A listing showing all MedDRA coding information will be prepared as well.

3.2 CLINICAL LABORATORY EVALUATION

3.2.1 Available data

Per protocol, the following laboratory parameters are expected:

- Serum folate
- Biochemistry: sodium, potassium, chloride, bicarbonate, urate, inorganic phosphate, creatinine, albumin, glucose, AST, ALT, alkaline phosphatase (ALP), gamma glutamylaminotransferase (GGT), lactate dehydrogenase (LDH), total and direct bilirubin, total serum proteins, blood urea nitrogen (BUN), C-reactive protein (CRP), creatine phosphokinase (CPK) and troponin I (Cohorts 2 and 3 only);
- Haematology: haemoglobin, haematocrit, red blood cell (RBC) count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count, platelet count, reticulocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes;
- Urinalysis: dipstick for glucose, protein, nitrite, pH and occult blood; microscopic examination for WBC, RBC and casts

Normal ranges are available as provided by the laboratory.

3.2.2 Derivation rules

The following abnormality categories will be defined:

- Low: value < lower limit of normal range
- Normal: lower limit of normal range ≤ value ≤ upper limit of normal range
- High: value > upper limit of normal range

Note:

- Classification will be done in standardised units, using non imputed values and limits.
- For the worst-case analysis visit, as defined in section 5.2.5, an additional category low + high is defined in case there are both low and high values.

Additionally, for liver enzymes, the following elevation categories will be defined:

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

3.2.3 Presentation of results

The statistical analysis will present results in standardised units.

Continuous laboratory parameters will be summarised by means of descriptive statistics at each analysis visit. Actual values and changes from baseline will be tabulated separately. Categorical and semi-quantitative parameters will be listed only.

Laboratory abnormalities will be presented as cross-tabulations of the abnormality at each post-baseline analysis visit and at the worst-case analysis visit versus the baseline abnormality. Numbers of subjects with treatment-emergent abnormalities will also be shown.

Additionally, a frequency table showing the liver enzyme elevation categories for the highest postdose values will be prepared.

All laboratory data will be listed. In addition, a listing will be provided showing only parameters for which abnormal values were reported.

Box plots of the actual values over time will be prepared for folates, the lab parameter of interest.

3.3 VITAL SIGNS

3.3.1 Available data

The following vital signs parameters are collected: supine heart rate, supine systolic (SBP) and diastolic blood pressure (DBP), oral body temperature and weight.

3.3.2 Derivation rules

Abnormalities are defined in below table.

	Pulse rate (bpm)	**SBP (mmHg)**	**DBP (mmHg)**	**Temp (°C)**
Low	<40	<90	<45	<35.0
Normal	40-100	90-150	45-90	35.0-37.5
High	>100	>150	>90	>37.5

Note: For the worst-case analysis visit, as defined in section 5.2.5, an additional category low + high is defined in case there are both low and high values.

3.3.3 Presentation of results

Vital signs parameters will be summarised by means of descriptive statistics at each analysis visit. Actual values and changes from baseline will be tabulated separately.

Abnormalities will be presented as cross-tabulations of the abnormality at each post-baseline analysis visit versus the baseline abnormality and as cross-tabulations of the worst-case abnormality versus the baseline abnormality. Numbers of subjects with treatment-emergent abnormalities will also be shown.

All vital signs data will be listed. In addition, a listing will be provided showing only abnormal values and their baseline.

Box plots of the actual values over time will be prepared for heart rate.

3.4 ELECTROCARDIOGRAMS

3.4.1 Available data

The following electrocardiogram (ECG) parameters will be collected: heart rate (HR), QRS interval, PR interval, QT interval and corrected QT interval (Bazett and Fridericia). Screening and predose recordings will be performed in triplicate.

3.4.2 Derivation rules

Mean values of the triplicates will be calculated per time point and rounded as detailed in section 5.3.4. Throughout the analysis, including the derivation of baseline and abnormalities, the mean values will be used. Individual triplicate values will only be listed.

Abnormalities for HR, QRS and PR interval are defined in below table.

	HR (bpm)	**PR (ms)**	**QRS (ms)**
Low	<40	<120	-
Normal	40-100	120-220	<=120
High	>100	>220	>120

Note: For the worst-case analysis visit, as defined in section 5.2.5, an additional category low + high is defined in case there are both low and high values.

For QTc interval (ms), the following categories are defined:

- Actual values:
  - ≤ 450 (normal)
  -]450; 480]
  -]480; 500]
  - > 500
- Changes:
  - ≤ 30 (normal)
  -]30; 60]
  - > 60

Note: The worst-case, as defined in section 5.2.5, is the highest value and associated change.

3.4.3 Presentation of results

Uncorrected QT interval will only be listed.

Continuous ECG parameters will be summarised by means of descriptive statistics at each analysis visit. Actual values and changes from baseline will be tabulated separately.

Abnormalities of the actual values will be presented as cross-tabulations of the abnormality at each post-baseline analysis visit, and at the worst-case analysis visit versus the baseline abnormality. Numbers and cumulative numbers (QTc only) of subjects with treatment-emergent abnormalities will also be shown.

Abnormalities of the QTc changes will be presented as tabulations of the change abnormality at each post-baseline analysis visit and at the worst-case analysis visit. Cumulative numbers of subjects with change abnormalities will also be shown.

All ECG data will be listed. In addition, a listing will be provided showing only abnormal values and their baseline.

Box plots of the actual values over time will be prepared for QTcFri.

3.5 PHYSICAL EXAMINATIONS

Abnormal physical examination findings will be listed.

4. GENERAL CHARACTERISTICS ANALYSES

4.1 SUBJECT DISPOSITION

The following subject data will be tabulated:

- The number of subjects in each analysis set, as defined in section 5.1.1 of this SAP
- Dates of first signed informed consent, last visit and last contact (overall only)
- The number and percentage of subjects who completed or discontinued the study as documented on the study termination page and the number and percentage of subjects for each study discontinuation reason

All information collected in the CRF concerning allocation, code breaking and study discontinuation and information on phases will be listed. The phases listing will also include the date of challenge and start and stop dates of rescue medication

4.2 PROTOCOL DEVIATIONS AND ELIGIBILITY

The number and percentage of subjects with major protocol deviations will be tabulated, overall and per class of deviation.

All available information concerning major protocol deviations, violations on eligibility criteria and subjects not treated will be listed.

4.3 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

4.3.1 Available data

The following parameters will be available in the clinical database:

- Demographics: gender, women of childbearing potential, age, race, ethnicity, height, weight at baseline, date of birth, date of signing informed consent form (ICF), alcohol consumption and smoking history
- Screening tests: serology, G6PD enzyme test, alcohol breath test and urine drug screen, pregnancy tests, standing heart rate and standing SBP and DBP (cohort 1 only), Beck depression inventory (question 9 - suicidal thoughts - and total score)

4.3.2 Derivation rules

The following parameters will be derived:

- Body mass index (BMI) at baseline (kg/m^2) = (weight at baseline (kg)) / (height (m))^2

  Note: The BMI will only be recalculated and rounded as detailed in section 5.3.4, when not available in the database.
- Smoking status:
  - Non smoker: SUOCCUR = N
  - Ex-smoker: SUOCCUR = Y and SUENRTPT = BEFORE
  - Smoker: SUOCCUR = Y and SUENRTPT = ONGOING

- Orthostatic change = value in supine position – value in standing position
- Beck depression inventory interpretation:
  - 0-10: Normal
  - 11-16: Mild mood disturbance
  - 17-20: Borderline clinical depression
  - 21-30: Clinical depression
  - 31-40: Severe depression
  - >40: Extreme depression

4.3.3 Presentation of results

Demographics will be presented using descriptive statistics for age, height, weight and BMI and frequency tabulations for gender, smoking status, race and ethnicity.

Orthostatic change and Beck depression inventory will be presented using descriptive statistics for the total score and frequency tabulations for interpretation.

All demographic data will be listed. Separate listings will also be created for Beck depression inventory, smoking history and alcohol consumption, serology and pregnancy tests (including EOS result).

4.4 MEDICAL HISTORY AND CONCOMITANT DISEASES

4.4.1 Available data

Medical history and concomitant diseases findings are coded using the medical dictionary for regulatory activities (MedDRA) into system organ classes and preferred terms. For each finding, a start and stop date or ongoing flag is collected.

4.4.2 Derivation rules

The following parameters will be derived:

- Medical history finding: not ongoing at screening (MHENRTPT = SCREENING)
- Concomitant disease finding: still ongoing at screening (MHENRTPT = ONGOING)

4.4.3 Presentation of results

Medical history and concomitant diseases will be tabulated separately. Each table will show:

- The number and percentage of subjects with and without findings
- The number and percentage of subjects with findings by system organ class and preferred term

All medical history and concomitant diseases data will be listed separately.

4.5 PRIOR AND CONCOMITANT THERAPIES AND RESCUE MEDICATION

4.5.1 Available data

All therapies (excluding rescue medication) are coded using WHO-DRUG. ATC selection is performed. ATC coding up to level 4 is available in the clinical database. For each therapy, a start date(time) and stop date(time) or ongoing flag are collected.

For rescue medication (cohorts 2 and 3) the start and end date(times) and the dose will be recorded.

4.5.2 Derivation rules

Based on their start and stop date(time), prior and concomitant therapies will be allocated to each phase during which they were administered. A therapy can therefore be reported in more than one phase.

Phases are defined in section 5.2.1. Therapies with (partially) missing dates will be allocated to each phase unless the available parts of the therapy start or stop date(time) provide evidence the therapy was not taken during that phase.

4.5.3 Presentation of results

The number and percentage of subjects with and without concomitant therapies and the number and percentage of subjects with therapies by generic term will be tabulated by phase (screening / treatment / rescue) and overall (treatment + rescue - challenge cohorts only).

The number and percentage of subjects with and without concomitant therapies and the number and percentage of subjects with therapies by ATC class (level 1, 2 and 4) and generic term will be tabulated by phase (screening / treatment / rescue) and overall (treatment + rescue - challenge cohorts only).

For the challenge cohorts 2 and 3, a table will be prepared showing the timing of rescue medication (prior to day 28 / on day 28) and the generic name and dose (malarone 250/100 mg / riamet 20/120 mg / primaquine 30 mg).

All prior and concomitant therapies data will be listed. A separate listing will be prepared for rescue medication.

4.6 EXPOSURE TO STUDY DRUG AND CHALLENGE AGENT

4.6.1 Available data

For all study drug administrations, the start date(times) and the dose will be recorded. For the challenge agent (cohorts 2 and 3) the start and end date(times) and the dose will be recorded.

4.6.2 Derivation rules

Not applicable

4.6.3 Presentation of results

All exposure and challenge data will be listed.

5. GENERAL METHODOLOGY

5.1 ANALYSIS SETS

5.1.1 Analysis sets

The following analysis sets will be considered in the statistical analysis:

All screened subjects set (SCR):	all subjects who *signed an informed consent* to participate in this study
All randomised subjects set (RND):	all subjects who were *randomised* into this study
Safety analysis set (SAF):	all subjects who were *exposed* to the study drug
PD analysis set:	*all randomised* subjects who were *exposed* to the study drug, have at least one evaluable PD data and no major protocol deviation affecting PD and who received the PfSPZ challenge inoculation (for cohort 2 and 3 only)
PK analysis set:	all randomised isubjects who have received at least 1 dose of P218, have at least one evaluable PK data and no major protocol deviation affecting PK

Programmer notes:

- Having signed an informed consent is defined as having a complete informed consent signature date in the database.
- Randomised is defined as having a complete randomisation date in the database or any information to confirm randomisation.
- Being exposed to the study drug is defined as having a complete exposure date in the database or any information to confirm an administration.
- The PK analysis set will be defined in the PK data review document, as elaborated in section 5.3.5. The major deviations and impact on analysis set will be determined prior to unblinding.

Unless stated otherwise, the SAF will be used for the general, safety and tolerability tables, listings and figures (TLFs). The PD set will be used for the pharmacodynamic TLFs and the PK set will be used for the analyses of PK data.

5.1.2 As planned versus as actual analysis

For all analyses, the actual treatment of the subject will be considered.

5.2 PHASES AND TIME POINTS

5.2.1 Phases

All events and assessments will be allocated to phases.

Phase	Start	End
Screening	Date of signing the ICF, with 00:00 added as time part	First administration date(time) – 1 minute
Treatment	First administration date(time)	First administration date(time) of rescue medication - 1 minute
Rescue	First administration date(time) of rescue medication	Date of last contact, with 23:59 added as time part

Rescue phase is not applicable for cohort 1. Per definition, and for each subject, the first phase starts on the date of the earliest available ICF signature, and the last available phase ends on the date of last contact, with 23:59 added as time part.

AEs and concomitant medications will be allocated to phases as described in sections 3.1.2 and 4.5.2 respectively. All other assessments will be allocated to phases based on the assessment date(time).

In case of (partially) missing date(time) fields, the visit label will be used to allocate to the correct phase. If this is not possible (unscheduled visits or visits on a turning point between phases), assessments will be allocated to the treatment phase unless the available parts of the assessments start or stop date(time) provide evidence for allocating to the screening/rescue phase.

5.2.2 Baseline and change from baseline

The baseline value is the last available and non-missing value before the first administration of any study drug. If all values are missing, the baseline will be reported as missing.

Wherever in this document 'change from baseline' is mentioned, it concerns the absolute change from baseline, defined as:

Absolute change from baseline at time point t = value at time point t – baseline value.

5.2.3 Relative day

Relative days (DY) will be calculated according to the following rule:

- Concerned date < reference date: DY = concerned date – reference date
- Concerned date ≥ reference date: DY = concerned date – reference date + 1

The reference date is the date of first administration of any study drug (cohort 1) / the date of inoculation (cohorts 2 and 3).

5.2.4 Analysis visits

For assessments after the reference date, the analysis will use the visits and time points indicated on the subject's case report form (CRF). Unscheduled assessments after the reference date will only be listed.

The screening value is the last available and non-missing value before day -1. This value corresponds to the screening visit, except in case of retesting. Reason for this approach is the use of retest results for subject eligibility assessment.

Baseline is defined in section 5.2.2.

5.2.5 Worst-case visit

A worst-case analysis visit will be created to summarise values considered as the worst-case. For abnormalities it is derived per parameter and in case both the lowest and the highest values are considered abnormal, a subject can have two worst-case analysis visits for a same parameter, which will be shown in tables as a combined high+low category. For malaria signs and symptoms, the worst-case corresponds to the highest score and is also derived per sign/symptom. *Note to François: would prefer to keep wording of two analysis visits, as this is how it will be in ADLB. Details added to show how it will be in table.*

All non-missing post-baseline values, including unscheduled assessments and follow-up will be considered when deriving the worst-case analysis visit. For challenge cohorts 2 and 3, worst-case will be derived both by phase (treatment/rescue) as relevant and overall.

5.3 IMPUTATION AND ROUNDING RULES

5.3.1 Missing values

For the calculation of the malaria clinical score, missing individual signs and symptom scores will be imputed with the mean of all non-missing scores.

For the pharmacokinetic analysis, if the first administration (Day 1) predose value is missing, then it will be set to 0 for the calculation of PK parameters (also see section 2.2.2).

5.3.2 Handling partially or completely missing dates in calculations

Not applicable

5.3.3 Values below or above a threshold

Safety values expressed as below (or above) the detection limit will be imputed by the value of the detection limit itself. Listings will always show the non-imputed values.

PK concentrations below the quantification limit will be flagged as Below the Limit of Quantification (BLQ) in the concentration tables. All BLQ values will be considered as 0.00 for PK and descriptive statistical analyses.

5.3.4 Rounding of derived variables

Derived variables will be rounded to the appropriate number of significant digits (see Definition of terms) at TLF level:

- Malaria clinical score will be rounded to 1 decimal.
- Mean of triplicates, mean scores and BMI will be rounded to 1 decimal.

All derivations will be done before rounding. Rounding will be done using the round half to up rule (see Definition of terms).

5.3.5 Outliers

For safety and PD, there will be no outlier detection. All measured values will be included in the analyses.

For PK values, it will be decided whether extreme values are to be excluded from the analysis based on a detailed data review performed by SGS PK after the data base lock and before performing the PK analysis.

Clinical deviations that could affect the PK data of a subject, as well as the observed abnormal drug levels, sampling time deviations and their impact on PK population/analysis will be discussed with the sponsor during this data review process, to decide keeping or excluding data points/subjects from PK population/analysis.

The PK Data Review document, detailing such kind of decisions, will be agreed and signed by both parts before running the final PK analysis.

5.4 PRESENTATION OF RESULTS

5.4.1 Calculation of descriptive statistics and percentages

For continuous parameters, full descriptive statistics will only be presented if there are at least 2 non-missing observations. Alternatively, only the number of non-missing data points and mean are shown.

Descriptive statistics for non-PK data will include the number of non-missing data points, the arithmetic mean, the standard deviation (SD), the median, minimum and maximum.

Descriptive statistics of the primary endpoint will additionally include the geometric mean and the corresponding 90% confidence interval (CI). Geometric mean and CI are calculated as the exponentials of the arithmetic mean and corresponding CI (based on t-distribution, without continuity correction) of the log-transformed data.

Descriptive statistics of PK concentrations will include the number of subjects with data, the number of subjects with BLQ data, arithmetic mean, SD, coefficient of variation (CV) % of arithmetic mean, median, minimum and maximum.

Descriptive statistics on PK parameters will additionally include geometric mean and geometric CV% (= 100 x $\sqrt{e^{(standard\ deviation\ of\ ln-transformed\ data)^2} - 1}$), except t_{max}: descriptive statistics will include the number of non-missing data points, the median, minimum and maximum. The descriptive statistics will be presented with 3 significant digits, except values >1000, which will be presented without decimals and t_{max} which will be presented with 2 decimals.

For event-type data, the denominator will be all subjects in the analysis set. All treatments will be shown, even if no events are present.

For frequency tabulations and cross-tabulations, missing values will not be included in the denominator count when computing percentages. For cross-tabulations of post-baseline results versus baseline results, a ‘missing’ category will be shown for baseline results if applicable. For the frequency tabulation of positive parasitaemia, two-sided 90% exact Clopper-Pearson confidence limits will be added.

5.4.2 Presentation of treatments

The following treatment labels will be used in the TLFs (excluding PK):

- Cohort 1:
  - Placebo
  - P218 1000 mg
- Challenge cohorts 2 and 3:
  - Placebo
  - P218 100 mg
  - P218 1000 mg
  - P218 total (only for AE tables)

In the PK analysis, the following treatment labels will be used in the TLFs:

- Cohort 1:
  - P218 1000 mg
- Challenge cohort 2:
  - P218 1000 mg
- Challenge cohort 3:
  - P218 100 mg

5.4.3 Ordering in tables, figures and listings

All general and safety tables and figures will be presented per part and treatment, unless specified otherwise. The following parts are defined:

- Cohort 1
- Challenge cohorts 2 and 3

All PD tables and figures will be presented per treatment, unless specified otherwise.

All PK tables and figures will be presented per cohort, treatment and administration day, unless specified otherwise.

All listings will be ordered by cohort, treatment, subject, analysis visit and time point, unless specified otherwise.

6. CHANGES TO THE PLANNED ANALYSIS

6.1 CHANGES NOT COVERED BY PROTOCOL AMENDMENTS BEFORE DATABASE LOCK

The protocol states placebo subjects will be pooled in tables. This can however not be done in all tables, as cohort 1 was not challenged and different cohorts have different assessment time points.

As the definitions of ITT and EFF set are identical, only the EFF set was retained. On request of the sponsor, PD is used rather than efficacy.

No AE tabulation by severity is foreseen in the present SAP. As this is a healthy volunteers study with a limited number of subjects and as malaria related symptoms are not captured as AEs, not many AEs are expected and a table by severity would be of limited added value. As an alternative, a table of grade ≥ 3 AEs is added.

The protocol mentions 1st and 3rd quartiles will be shown in the descriptive tables. However, the sponsor doubts these will be of added value and it was decided not to show them to improve readibility of the tables.

6.2 CHANGES NOT COVERED BY PROTOCOL AMENDMENTS AFTER DATABASE LOCK

Not applicable

6.3 CHANGES TO THE FINAL STATISTICAL ANALYSIS PLAN

Not applicable

7. REFERENCES

ICH Topic E6(R2) Guideline for Good Clinical Practice – Step 4, 9 November 2016.

ICH Topic E9 Statistical Principles for Clinical Trials – Step 5 – Note for Guidance on Statistical Principles for Clinical Trials (CPMP/ICH/363/96), September 1998.

8. LIST OF TABLES, LISTINGS AND FIGURES

8.1 TABLES

14.3.1.5	Non-serious Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Term	SAF
14.3.1.6	Grade 3 or More Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Term	SAF
14.3.1.7	P218 Related Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Term	SAF
14.3.1.8	PfSPZ Inoculum Related Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Term	SAF
14.3.1.9	Procedure Related Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Term	SAF
14.3.1.10	Rescue Drug Related Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Term	SAF
14.3.1.11	Serious P218 Related Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Term	SAF
14.3.1.12	Treatment-Emergent Adverse Events for which the Study Was Discontinued by MedDRA System Organ Class and Preferred Term	SAF
14.3.1.13	Treatment-Emergent Adverse Events for which the Study Drug Was Discontinued by MedDRA System Organ Class and Preferred Term	SAF
LABORATORY DATA		
14.3.2.1	Descriptive Statistics of Laboratory Test Results	SAF
14.3.2.2	Cross-Tabulation of Laboratory Abnormalities Versus Baseline	SAF
14.3.2.3	Liver Enzyme Elevation Categories	SAF
VITAL SIGNS		
14.3.3.1	Descriptive Statistics of Vital Signs	SAF
14.3.3.2	Cross-Tabulation of Vital Signs Abnormalities Versus Baseline	SAF
ECG		
14.3.4.1	Descriptive Statistics of ECG	SAF
14.3.4.2	Cross-Tabulation of ECG Abnormalities Versus Baseline	SAF
14.3.4.3	Tabulation of QTc Change Abnormalities	SAF

8.2 LISTINGS

GENERAL CHARACTERISTICS		
16.2.1.1	Allocation	RND
16.2.1.2	Code Breaking Information	RND
16.2.1.3	Analysis Phases	RND
16.2.1.4	Study Discontinuation	RND
16.2.2.1	Protocol Deviations	RND
16.2.2.2	Violations on Eligibility Criteria	RND
16.2.2.3	No-Treatment Subjects	SCR minus SAF
16.2.2.4	Subjects Excluded from Any Analysis Sets	SCR

16.2.4.1	Demographic Data	SAF
16.2.4.2	Smoking History and Alcohol Consumption	SAF
16.2.4.3	Orthostatic Change in Vital Signs at Screening	SAF
16.2.4.4	Beck Depression Inventory at Screening	SAF
16.2.4.5	Screening Tests	SAF
16.2.4.6	Pregnancy Tests	SAF
16.2.4.7	Medical History	SAF
16.2.4.8	Concomitant Diseases	SAF
16.2.4.9	Prior and Concomitant Therapies	SAF
16.2.4.10	Rescue Medication	SAF
16.2.4.11	Comments	SAF
16.2.5.1	Exposure and Challenge Data	SAF
PHARMACOKINETICS		
16.2.5.2	PK Data Handling	PK
16.2.5.3	Actual Blood Sampling Times for PK Assessments (h)	PK
PHARMACODYNAMICS		
16.2.6.1	Parasitaemia	PD
16.2.6.2	Malaria Signs and Symptoms	PD
SAFETY		
ADVERSE EVENTS		
16.2.7.1	Adverse Events	SAF
16.2.7.2	Serious Adverse Events	SAF
16.2.7.3	Fatal Adverse Events	SAF
16.2.7.4	Treatment Emergent Adverse Events for Which the Study Were Discontinued	SAF
16.2.7.5	Treatment Emergent Adverse Events for Which the Study Drug Were Discontinued	SAF
16.2.7.6	Treatment Emergent Adverse Events After Administration of Rescue Drug	SAF
16.2.7.7	Adverse Events: Coding Information	SAF
16.2.7.8	Physical Examinations Abnormalities	SAF
LABORATORY DATA		
16.2.8.1	Laboratory Test Results	SAF
16.2.8.2	Abnormal Laboratory Test Results	SAF
VITAL SIGNS		
16.2.9.1	Vital Signs Results	SAF
16.2.9.2	Abnormal Vital Signs Results	SAF
ECG		
16.2.10.1	ECG Results	SAF
16.2.10.2	Abnormal ECG Results	SAF

16.2.10.3	ECG Interpretation and Morphology for Subjects with any Abnormal Interpretation	SAF

8.3 FIGURES

PHARMACODYNAMICS

14.2.1.1	Median Parasitaemia Actual Values Over Time	PD
14.2.1.2	Kaplan-Meier Plot of Time to Positive Parasitaemia	PD
14.2.1.3	Mean (SE) Malaria Clinical Score Actual Values Over Time	PD
16.2.6.1	Individual Parasitaemia Actual Values Over Time	PD
16.2.6.2	Individual Malaria Clinical Score Actual Values Over Time	PD

PHARMACOKINETICS

14.2.2.1	Mean (SD) P218 Plasma Concentration vs. Time Profiles	PK
14.2.2.2	Mean (SD) P218 beta-acyl-glucuronide Plasma Concentration vs. Time Profiles	PK
14.2.2.3	Mean (SD) P218-OH beta-acyl-glucuronide Plasma Concentration vs. Time Profiles	PK
14.2.2.4	Mean (SD) P218-OH Plasma Concentration vs. Time Profiles	PK
16.2.5.1	Individual P218 Plasma Concentration vs. Time Profiles, per Cohort	PK
16.2.5.2	Individual P218 beta-acyl-glucuronide Plasma Concentration vs. Time Profiles, per Cohort	PK
16.2.5.3	Individual P218-OH beta-acyl-glucuronide Plasma Concentration vs. Time Profiles, per Cohort	PK
16.2.5.4	Individual P218-OH Plasma Concentration vs. Time Profiles, per Cohort	PK
16.2.5.5	Individual P218 Plasma Concentration vs. Time Profiles, per Subject	PK
16.2.5.6	Individual P218 beta-acyl-glucuronide Plasma Concentration vs. Time Profiles, per Subject	PK
16.2.5.7	Individual P218-OH beta-acyl-glucuronide Plasma Concentration vs. Time Profiles, per Subject	PK
16.2.5.8	Individual P218-OH Plasma Concentration vs. Time Profiles, per Subject	PK

LABORATORY DATA

14.3.2.1	Box plots of Folate Actual Values Over Time	SAF

VITAL SIGNS

14.3.3.1	Box plots of Heart Rate Actual Values Over Time	SAF

ECG

14.3.4.1	Box plots of QTcFri Actual Values Over Time	SAF

9. APPENDICES

9.1 SAS CODE

Not applicable

9.2 ADVERSE EVENTS OF SPECIAL INTEREST

Hepatic

- Any ALT or AST above 3x ULN;
- Any elevation in bilirubin 2x ULN;
- Any AST or ALT above 2x ULN and (total bilirubin level (TBL) >1.5x ULN or INR >1.4);
- Any AST or ALT above 2x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (eosinophil percent or count above the ULN).

Cardiac

- QTcB or QTcF at any time >480 ms;
- Bundle branch block (except right bundle branch block that was present prior to IMP administration);
- Any arrhythmia, except:
  - sinus bradycardia that is clinically asymptomatic, and not associated with any other relevant ECG abnormalities;
  - sinus tachycardia that is clinically asymptomatic, and associated with a body temperature >38.0 °C, and not associated with any other relevant ECG abnormalities;
  - respiratory sinus arrhythmia;
  - wandering atrial pacemaker;
  - isolated, single premature atrial/ventricular complex (i.e., no bigeminy, trigeminy, couplets, triplets or salvos) that does not occur more than once in a particular ECG tracing.

Haematological

- Haemoglobin drop >2 g/dL and under lower limit of normal from baseline prior to inoculation;
- Absolute neutrophil count <1000/µL;
- Platelet count <100,000 /mm3.

*Dermatological:**

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

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

9.3 SCHEDULE OF ASSESSMENTS

9.3.1 Cohort 1

Description	**SCR**	**Treatment and follow-up**																							**EOS**
Study Day	**-28 to -2**	**-1[a]**	**1**						**2**		**3**								**4**		**5**	**6**	**7**	**8**	**9**
Timepoint (h) in relation to 1st IMP administration		**0**	**0[b]**	**1**	**2[b]**	**4**	**6**	**10**	**24**	**36**	**48**	**48.5**	**49**	**49.5**	**50**	**52**	**54**	**58**	**72**	**84**	**96[c]**	**120[c]**	**144[c]**	**168[c]**	**192[c]**
Ambulatory visit	X																				X	X	X	X	X
Confinement in clinical unit[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Eligibility criteria	X	X																							
Informed consent[o]	X																								
Demographics	X																								
Medical and social history	X																								
Beck depression inventory	X																								
Randomisation[p]			X																						
Alcohol & drug screen[e]	X	X																							
Height & weight[f]	X	X																		X					X

Description	**SCR**	**Treatment and follow-up**																						**EOS**
Study Day	**-28 to -2**	**-1[a]**	**1**						**2**		**3**								**4**		**5**	**6**	**7**	**8**	**9**
Timepoint (h) in relation to 1st IMP administration		**0**	**0[b]**	**1**	**2[b]**	**4**	**6**	**10**	**24**	**36**	**48**	**48.5**	**49**	**49.5**	**50**	**52**	**54**	**58**	**72**	**84**	**96[c]**	**120[c]**	**144[c]**	**168[c]**	**192[c]**
Physical examination[g]	X	X							X										X						X
Vital signs[h]	X	X	X	X	X			X	X		X				X			X	X	X		X			X
12-lead ECG[i]	X	X	Xi						X		Xi								X			X			X
Serology[j]	X																								
Pregnancy test[k]	X	X																							X
IMP administration[l]			X								X														
Haematology, chemistry & urinalysis[q]	X	X									X											X			X
Serum folate	X	X[r]									X											X			X
Coagulation parameters[m]	X	X									X											X			X
PK blood sample[n]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Xn	X	X	X	X	X
Previous medications	X																								
Concomitant medications		X---------- X																							

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

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

[a] Assessments begin as of subject confinement in the clinical unit and must be completed and outcomes must be available before IMP administration.

[b] The assessments indicated must be completed and outcomes must be available before IMP administration. Predose PK sample should be taken within 1 hour before IMP administration.

[c] The assessments indicated will be performed daily during ambulatory visits to the clinical unit.

[d] Subjects will be admitted to the clinical unit in the morning of Day -1 and will remain in confinement until Day 4, i.e. 36 hours after second IMP administration.

[e] Alcohol breath test and urine dipstick screening for drug abuse.

[f] Height to be measured at screening only; body weight to be measured at screening, upon admission to the clinical unit on Day-1, upon discharge from the clinical unit on Day 4 and at the EOS visit on Day 9.

[g] Full physical examination will be conducted at screening and at the EOS visit on Day 9. Targeted (symptom-driven) physical examination will be conducted on all other occasions. Symptom-driven physical examination will be conducted at any time during follow-up, if indicated.

[h] Vital signs (blood pressure, pulse and body temperature) will be measured after remaining 5 minutes in a supine position. At screening, orthostatic changes to blood pressure and pulse rate will also be assessed: subjects will be requested to stand after completion of the supine measurements and blood pressure and pulse rate will be recorded after 2 min in the standing position.

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

[j] Serological testing for human immunodeficiency virus (HIV) antibody and antigen, hepatitis B surface antigen (HbsAg) and anti-hepatitis C virus (HCV) antibody, to determine eligibility for the trial.

[k] Pregnancy testing consists of serum β-human chorionic gonadotropin (β-HCG) assessment at screening and urine β-HCG assessments on Day-1 and at the EOS visit.

[l] In each instance, the subject will receive a single oral dose of 1000 mg P218 or placebo after fasting at least 8 hours.

[m] International Normalised Ratio (INR), prothrombin time (PT) and activated partial thromboplastin time (aPTT).

[n] On Day 4, the subject will be discharged from the clinical unit only after the PK sample at 84 hours is drawn.

[o] No study-related procedure is to be performed before voluntarily signing of the informed consent form.

[p] Randomization will be performed before first IMP administration.

[q] Laboratory tests will be performed after fasting for at least 8 hours.

[r] This assessment can be done at either D-3 or D-1, upon Investigator's decision.

9.3.2 Cohort 2 and 3

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

Description	**SCR**	**Challenge, treatment and follow-up**																																		**EOS visit**	**EOS[u]**
Study Day	**-28 to -2**	**-1[a]**	**1**									**2**		**3**								**4**		**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14-28[c]**	**35**	**45 max**
Timepoint (h) in relation to PfSPZ Challenge		**0**	**0[b]**	**2[b]**	**2.5**	**3**	**3.5**	**4**	**6**	**8**	**12**	**24**	**36**	**50**	**50.5**	**51**	**51.5**	**52**	**54**	**56**	**60**	**72**	**84**	**96**	**120**	**144**	**168**	**192**	**216**	**240**	**264**	**288**			
G6PD deficiency enzyme test	X																																			
Serology[k]	X																																			
Pregnancy test[l]	X	X																																	X	
DVI of PfSPZ Challenge			X																																	
IMP administration[m]				X										X																							
Malaria clinical score[n]			X																							X	X	X	X	X	X	X	X	X		
Haematology, chemistry & urinalysis[o]	X	X												X													X		X				X[o]	X	
Serum folate	X	X[v]												X													X		X				X[o]	X	
Coagulation parameters[p]	X	X												X													X									
PK blood sample			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X								
Rescue therapy[q]																																		X		
TBS[r]																										X	X	X	X	X	X	X	X	X		

Description	SCR	Challenge, treatment and follow-up																																	EOS visit	EOS[u]
Study Day	**-28 to -2**	**-1[a]**	**1**									**2**		**3**								**4**		**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14-28[c]**	**35**	**45 max**
Timepoint (h) in relation to PfSPZ Challenge		**0**	**0[b]**	**2[b]**	**2.5**	**3**	**3.5**	**4**	**6**	**8**	**12**	**24**	**36**	**50**	**50.5**	**51**	**51.5**	**52**	**54**	**56**	**60**	**72**	**84**	**96**	**120**	**144**	**168**	**192**	**216**	**240**	**264**	**288**			
qPCR for parasites[s]			X																							X	X	X	X	X	X	X	X	X	
Previous medications	X																																			
Concomitant medications		X----------X																																		
AEs		X----------X																																		
SAE reporting	X----------X																																			

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

[a] Assessments begin as of subject confinement in the clinical unit and must be completed and safety assessment outcomes must be available before PfSPZ Challenge DVI on Day 1.

[b] On Day 1, assessments begin as of 3 hours before challenge. The assessments indicated must be completed and safety assessment outcomes must be available before PfSPZ inoculation or before IMP administration. Predose PK sample should be taken within 1 hour before IMP administration.

[c] The assessments indicated will be performed daily during ambulatory visits to the clinical unit.

[d] Subjects will be admitted to the clinical unit in the morning of Day -1 and will remain in confinement until Day 13, i.e. 12 days post PfSPZ Challenge.

[e] No study-related procedure is to be performed before voluntarily signing of the informed consent form.

[f] Alcohol breath test and urine dipstick screening for drug abuse.

[g] Height to be measured at screening only; body weight to be measured at screening, upon admission to the clinical unit on Day-1, upon discharge from the clinical unit on Day 13 and at the EOS visit on Day 35.

[h] Full physical examination will be conducted at screening, at Days -1, 2, 4, 8 and 13, and at the EOS visit on Day 35. Targeted (symptom-driven) physical examination will be conducted on Days 5, 10, 15, 20 and 25, and at the start of rescue treatment. Symptom-driven physical examination will be conducted at any time during follow-up, if indicated.

[i] Vital signs (blood pressure, pulse and body temperature) will be measured after remaining 5 minutes in a supine position. During the period between Day 14 and 28, vital signs will be measured on Days 15, 20 and 25 and on the first day of rescue treatment only.

[j] 12-lead ECGs recordings will be performed after subjects have remained in a supine position for at least 10 minutes. All recordings will be performed once, except at the screening and before the first and second IMP administration when they will be performed in triplicates. During the period between Day 14 and 28, one record will be performed on the first day of rescue treatment only.

[k] Serological testing for HIV antibody and antigen, HbsAg and anti-HCV antibody, to determine eligibility for the trial.

[l] Pregnancy testing consists of serum β-HCG assessment at screening and urine β-HCG assessments on Day-1 and at the EOS visit.

[m] In each instance, the subject will receive a single oral dose of 1000 mg P218 or placebo (Cohort 2) or a single oral dose of 100 mg P218 or placebo (Cohort 3) after fasting 8 hours at least. The initial dose at Day 1 must not be given prior to 2 hours post-inoculation.

[n] Malaria clinical score for malaria signs and symptoms is assessed on Day 1, in the 3 hours before PfSPZ Challenge, and daily from Day 7 to the day subject is positive for parasitaemia and at the EOS visit at Day 35. Daily assessments will take place during rescue treatment. For subjects that not develop positive parasitaemia until Day 28, after Day 28, assessments will take place only at the EOS visit at Day 35.

[o] During the period between Day 14 and Day 28, haematology, clinical chemistry and urinalysis laboratory tests will be performed and serum folate measured after fasting for at least 8 hours on Days 14, 21 and 28, and additionally one day after the subject turned positive for parasitaemia, if not coincident with one of these scheduled timepoints. Troponin I will be measured at screening, baseline prior to inoculation and on Days 2 and 3 of rescue treatment.

[p] INR, PT and aPTT.

[q] Every subject will receive a three-day course of antimalarial rescue medication as soon as positive for parasitaemia or on Day 28 in subjects who do not develop positive parasitaemia throughout the follow-up period. On the first day of rescue treatment, the subject will also receive a single oral dose of Primaquine® to ensure complete clearance of gametocytes.

[r] Blood samples for the assessment of parasitaemia by TBS microscopy will be drawn daily from Day 7 to Day 28. In subjects who develop parasitaemia and receive rescue treatment, TBS microscopy will be performed daily for confirmation of the qPCR outcomes until treatment completion and success, and at the EOS visit on Day 35. In subjects who do not develop positive parasitaemia until Day 28 of the study, rescue treatment will be initiated on Day 28. Instead of daily qPCR and TBS assessments until treatment success, for these subjects only a final qPCR will be performed at the EOS visit on Day 35.

[s] Blood samples for the assessment of parasitaemia by qPCR will be drawn at the following timepoints: immediately before PfSPZ Challenge DVI and daily from Day 7 to Day 28. In subjects who develop parasitaemia and receive rescue treatment, qPCR will be performed daily until treatment completion and one negative outcome is obtained, and at the EOS visit on Day 35. In subjects who do not develop positive parasitaemia until Day 28 of the study, rescue treatment will be initiated on Day 28. Instead of daily qPCR and TBS assessments until treatment completion and success, for these subjects only a final qPCR will be performed at the EOS visit on Day 35.

[t] Randomisation will be performed before first IMP administration.

[u] All subjects that receive rescue therapy will be asked non leading questions to determine the occurrence of any AEs approximately two weeks after initiation of the rescue treatment, either during a planned study visit or by phone in case there is no planned study visit at that time. If this time point is after the EOS visit on Day 35, the moment of the phone call will be considered the EOS and not Day 35.

[v] This assessment can be done at either D-3 or D-1, upon Investigator's decision.